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Cytogenetic and molecular delineation of the smallest commonly deleted region of chromosome 5 in malignant myeloid diseases

(myeloid leukemias/tumor-suppressor genes/fluorescence *in situ* hybridization/therapy-related leukemia)

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ABSTRACT **Loss of a whole chromosome 5 or a deletion of its long arm (5q) is a recurring abnormality in malignant myeloid neoplasms. To determine the location of genes on 5q that may be involved in leukemogenesis, we examined the deleted chromosome 5 homologs in a series of 135 patients with malignant myeloid diseases. By comparing the breakpoints, we identified a small segment of 5q, consisting of band 5q31, that was deleted in each patient. This segment has been termed the critical region. Distal 5q contains a number of genes encoding growth factors, hormone receptors, and proteins involved in signal transduction or transcriptional regulation. These include several genes that are good candidates for a tumor-suppressor gene, as well as the genes encoding five hematopoietic growth factors (*CSF2, IL3, IL4, IL5,* and *IL9*). By using fluorescence *in situ* hybridization, we have refined the localization of these genes to 5q31.1 and have determined the order of these genes and of other markers within 5q31. By hybridizing probes to metaphase cells with overlapping deletions involving 5q31, we have narrowed the critical region to a small segment of 5q31 containing the *EGR1* gene. The five hematopoietic growth factor genes and seven other genes are excluded from this region. The *EGR1* gene was not deleted in nine other patients with acute myeloid leukemia who did not have abnormalities of chromosome 5. By physical mapping, the minimum size of the critical region was estimated to be 2.8 megabases. This cytogenetic map of 5q31, together with the molecular characterization of the critical region, will facilitate the identification of a putative tumor-suppressor gene in this band.**

Recurring chromosomal rearrangements are characteristic of human malignant diseases, particularly the leukemias and lymphomas (1). The major emphasis of the molecular analysis of the chromosomal abnormalities in human tumors has involved the recurring translocations, in which two gene sequences are juxtaposed, resulting in the activation of an oncogene in a dominant fashion. More recently, the loss of genetic material, resulting from chromosomal loss or deletion or from other mechanisms, has received considerable attention. The genetic consequence is the development of hemizygosity resulting in a gene dosage effect or in the unmasking of a recessive allele, such as a tumor-suppressor gene, on the cytogenetically "normal" homolog (2). The unmasked allele is usually abnormal in structure and/or function. Retinoblastoma is the prototypic model for the study of tumor-suppressor genes; however, they have been implicated in the pathogenesis of a number of other tumors for which allele loss or chromosome loss or deletion has been demonstrated (3).

The occurrence of a myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) is a late complication of cytotoxic therapy used in the treatment of both malignant and nonmalignant diseases (4). Therapy-related MDS or AML (t-MDS/t-AML) typically presents ≈5 years after treatment. Frequently, all three hematopoietic cell lines (erythroid, myeloid, and megakaryocytic) are involved in the myelodysplastic process. Survival times of patients with t-AML are usually short (median, 8 months).

At the cytogenetic level, t-MDS/t-AML is characterized by loss of an entire chromosome 5 or 7 or a deletion of the long arm of these chromosomes [del(5q)/del(7q)] (5, 6). In our recently updated series of 129 consecutive patients with t-MDS/t-AML (ref. 7; M.M.L.B. and R.A.L., unpublished work), 120 (93%) had a clonal chromosomal abnormality and 97 (75%) had loss or deletion of chromosome 5 and/or 7. Among these 97 patients, 21 had loss of chromosome 5, 26 had a del(5q), 8 had loss of 5q following unbalanced translocations, 51 had loss of chromosome 7, 11 had a del(7q), and 12 had loss of 7q as a result of an unbalanced translocation. Thirty-one patients had abnormalities of both chromosomes 5 and 7. Overall, 55 patients (43%) had abnormalities of chromosome 5. A del(5q) was the most common structural aberration in our series.

In addition to t-MDS/t-AML, a −5/del(5q) has also been observed in the malignant cells of 10% of patients with AML *de novo* and in 15% of patients who have MDS arising *de novo* (8). Many of these patients have had significant occupational exposure to potential environmental carcinogens, suggesting that abnormalities of chromosome 5 or 7 may be a marker of mutagen-induced leukemia. A distinct clinical syndrome associated with a del(5q) is seen in a subset of patients with MDS *de novo*. Clinically, this disorder, termed the "5q− syndrome," is characterized by refractory anemia (RA). These patients having a del(5q) as the sole abnormality tend to have a relatively mild course that usually does not progress to acute leukemia (9).

We and others have proposed that the long arm of chromosome 5 contains a myeloid tumor-suppressor gene and that this gene is likely to be located within a commonly deleted segment in patients who have a del(5q). By cytogenetic analysis of 135 patients with a del(5q), we have identified a commonly deleted segment or critical region. In addition, we have used fluorescence *in situ* hybridization (FISH) and pulsed-field gel electrophoresis (PFGE) to prepare a cytogenetic and physical map of this region of chromosome 5 and to identify DNA sequences located within the critical region.

MATERIALS AND METHODS

Patients. We examined 135 patients who were diagnosed and treated at the University of Chicago Medical Center or who were treated at other metropolitan Chicago hospitals and

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Abbreviations: AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; t-MDS/t-AML, therapy-related MDS or AML; RA, refractory anemia; FISH, fluorescence *in situ* hybridization; PFGE, pulsed-field gel electrophoresis; DAPI, 4′,6-diamidino-2-phenylindole; YAC, yeast artificial chromosome; cM, centimorgan(s); CEPH, Centre d'Etude du Polymorphisme Humaine.

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were referred to our cytogenetics laboratory between 1970 and 1991. The diagnosis and subclassification of MDS or AML were based on morphological and cytochemical studies of peripheral blood smears and bone marrow aspirates and biopsy specimens obtained prior to therapy, according to the French/American/British Cooperative Group criteria (10, 11). Cytogenetic analysis was performed with quinacrine fluorescence and trypsin–Giemsa banding techniques on bone marrow cells from aspirate or biopsy specimens or on peripheral blood cells obtained at the time of diagnosis. We examined metaphase cells from direct preparations or from short-term (24 and 48 hr) unstimulated cultures. Chromosomal abnormalities are described according to the International System for Human Cytogenetic Nomenclature.

DNA Probes. Cosmid clones were obtained from a library prepared from the somatic cell hybrid HHW141, which contains chromosome 5 as its only human chromosome (12). These clones were kindly provided by Yusuke Nakamura. The cosmids recognize restriction fragment length polymorphisms and were typed on 63 Centre d'Etude du Polymorphisme Humaine families (refs. 12 and 13; CEPH version 4). The clones were localized to chromosome 5 by FISH (14) and somatic cell hybrid mapping (15). Of these, 10 cosmid probes which mapped to bands 5q23.3–32 were chosen for additional mapping studies (Table 1). Cosmid DNA was prepared by using the Qiagen (Diagen, Düsseldorf, F.R.G.) maxi-preparation columns and protocols.

Two yeast artificial chromosome (YAC) clones which contained the *CSF2/IL3* (B221D4, 240 kb) and the *IL4/IL5/IRF1* genes (A94G6, 425 kb) were used for FISH (17). The *IRF1* gene is linked to the *IL5* gene and is contained in the A94G6 YAC. The yeast clones containing YACs were propagated, and high molecular weight yeast chromosomal DNA was prepared (17). The *EGR1* cosmid and *IL9* phage clones were kindly provided by M. Lovett (Gene Labs, Redwood City, CA) and Y.-C. Yang (Indiana University, Indianapolis), respectively. λ phage clones of the *TCF7, CD14, FGFA, GRL, SPARC*, and *ADRA1* genes and the *D5S89* locus were obtained by screening a phage library of flowsorted chromosome 5 homologs using genomic or cDNA probes (kindly provided by L. Deaven, Los Alamos National Laboratory, Los Alamos, NM).

FISH. FISH was performed as described (18). Biotin-labeled probes were prepared by nick-translation using Bio-11-dUTP (Enzo Diagnostics). Biotinylated probes were detected with fluorescein-conjugated avidin (Vector Laboratories). Cytogenetic map locations were determined by analyzing the location of the signal relative to the *Alu* R-banding pattern observed on hybridized chromosomes or the 4′,6-diamidino-2-phenylindole (DAPI) banding pattern or by viewing the hybridized cells with a quinacrine mustard filter set, which allows the simultaneous visualization of DAPI-stained chromosomes and the fluorescein signal. *Alu* R-banding was achieved by adjusting the concentration of competitor Cot1 DNA (GIBCO/BRL) in the hybridization mixture to result in weak hybridization of repetitive elements in the probe sufficient to induce faint R-bands.

For dual-color fluorescence, probes were labeled by nick-translation with Bio-11-dUTP or with digoxigenin-11-dUTP (Boehringer Mannheim). The biotin-labeled probes were detected with fluorescein-conjugated avidin. The digoxigenin-labeled probes were detected by incubation with rhodamine-conjugated sheep anti-digoxigenin antibodies (Boehringer Mannheim). Slides were examined with a fluorescein/rhodamine double-bandpass filter set (Omega Optical, Brattleboro, VT).

To determine the order of DNA probes by dual-color FISH, combinations of two probes were initially hybridized to metaphase or prometaphase cells prepared from mitogen-stimulated lymphocytes. The slides were examined by two independent observers (5–15 cells scored per observer) without knowledge of the probes used. The results were confirmed by repeating the hybridizations and by hybridizing different combinations of probes.

PFGE. The methods for restriction endonuclease digestions and electrophoresis (17) and for transfer of DNA to nylon membranes and hybridizations (19) were performed as described.

Table 1. DNA probes used for FISH

Probe	Locus/gene	FISH location	Previous location	Ref.
MC5.71	*D5S150*	5q23.3–31.1	5q	12–14
MC5.138	*D5S166*	5q31	5q	12–14
MC5.95	*D5S147*	5q31	5q	12–14
EF5.12	*D5S178*	5q31	5q	12–14
YN5.32	*D5S162*	5q31	5q	12–14
KK5.100	*D5S155*	5q31	5q	12–14
YN5.116	*D5S151*	5q31	5q	12–14
E5.21	*D5S156*	5q31	5q	12–14
WLN1	—	5q31	5q	12–14
cp12.6	*D5S89*	5q31	5q21–33.1	16
92.15	*D5S179*	5q32	5q	12–14
	IL3/CSF2	5q31.1	5q23 –31	16
	IL4/IL5/IRF1	5q31.1	5q23–32	16
	TCF7	5q31.1	5q31	16
	IL9	5q31.1	5q22–32	16
	EGR1	5q31.1	5q23–31	16
	CD14	5q31	5q23–31	16
	FGFA	5q31	5q31.3–33.2	16
	GRL	5q31	5q31–32	16
	SPARC	5q31.3–32	5q31–33	16
	ADRA1	5q33	5q32–34	16

RESULTS

Cytogenetic Delineation of the Critical Region. To determine the location of genes on 5q that may be involved in myeloid leukemogenesis, we previously examined the breakpoints and the extent of the deletions in 17 patients with t-MDS/t-AML (7). Our cytogenetic analysis revealed that these deletions were interstitial, with a proximal breakpoint commonly in q13–q15 and a distal breakpoint in q33–q35; bands q23 and q31 were deleted in each patient (7).

To refine the critical region of 5q, we have examined the breakpoints of the deletions in an expanded series of 135 patients. This analysis includes 33 patients who had t-MDS/t-AML, 85 patients who had *de novo* MDS or AML, and 17 patients who had the RA 5q− syndrome (Fig. 1). The proximal breakpoints were variable and were distributed in bands q11–q31. The most common proximal breakpoints were q13 (59 patients) and q15 (25 patients). Ninety patients had a distal breakpoint in 5q33.3; less often, distal breakpoints were observed in 5q31 (10 patients), 5q32 (1 patient), 5q34 (17 patients), or 5q35 (17 patients). For patients with

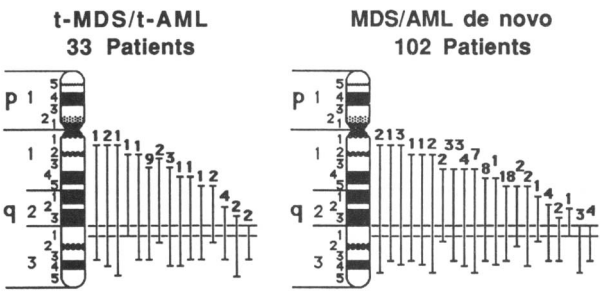

FIG. 1. Diagram of the banding pattern of chromosome 5 illustrating the breakpoints and deletions in 135 patients with myeloid disorders. The number above each vertical bar indicates the number of patients with this deletion. The horizontal lines at right delineate the smallest commonly deleted segment (5q31).

t-MDS/t-AML or *de novo* MDS/AML, the identification of patients who had a *proximal* breakpoint in q31 (9 patients) and of other patients who had a *distal* breakpoint in this band (10 patients) allowed us to refine the critical region of 5q to band q31. We have also identified four patients with AML who have balanced translocations involving 5q31, providing further support that the critical region of 5q may be limited to q31 (data not shown). Thus, it is likely that loss of a gene(s) located within this band is involved in the pathogenesis of myeloid disorders characterized by a del(5q).

Cytogenetic Map of 5q31. As an initial step in preparing a physical map of 5q31, we performed FISH of 49 cosmid clones from 5q (14). These clones were assigned to 5q by genetic linkage analysis (CEPH version 4); however, the chromosomal location of only 2 of these clones had been determined by physical mapping techniques. The results of these previous studies allowed us to localize all of these probes to discrete chromosomal band(s) and, more importantly, to identify 11 probes that were located within 5q31 (14). Nine of these probes were selected for additional studies (Table 1).

To determine the order of the nine cosmids, we used dual-color FISH. The level of resolution of probes is estimated to be 1–2 megabases (Mb) in metaphase cells and ≈25 kb in interphase cells (20). We also used FISH interactively with genetic linkage analysis to determine the order of these probes and to estimate the genetic distances between them (C.A.W., M.M.L.B., W.L.N., M. Mullan, and R. Williamson, unpublished data). The cosmid order and estimated distances between cosmids were as follows: cen–*D5S150*–6.7 cM–*D5S166*–8.9 cM–*D5S147*–1.8 cM–*D5S178*–0.1 cM–*D5S162*–4.2 cM–*D5S155*–7.3 cM–*D5S151*–8.7 cM–*D5S156*–*WLN1*–qter (cM, centimorgan; cen, centromere; qter, q-arm terminus). By genetic linkage analysis, *D5S178* and *D5S162* were 0.1 cM apart. These markers could be ordered by FISH when we examined extended prometaphase chromosomes, and were physically linked on a 100 kb *Sfi* I fragment (data not shown).

A striking number of genes encoding growth factors and growth factor receptors have been mapped to distal 5q, including the interleukin 3 (*IL3*), interleukin 4 (*IL4*), interleukin 5 (*IL5*), interleukin 9 (*IL9*), and granulocyte/macrophage-colony-stimulating factor (*CSF2*) genes at 5q23–31 and the macrophage-colony-stimulating factor receptor (*CSF1R*) gene at 5q33 (see ref. 16 for references). Other genes that have been mapped within or adjacent to 5q31 include the genes encoding the α_1- and β_2-adrenergic receptors (*ADRA1* and *ADRB2*, 5q31–34), endothelial cell growth factor (*FGFA*, 5q31–32), the CD14 myeloid antigen (5q23–31), the early growth response 1 protein (*EGR1*, 5q23–31), a T-cell specific transcription factor (*TCF7*, 5q31), osteonectin (*SPARC*, 5q31–33), and the glucocorticoid receptor (*GRL*, 5q31–32) (16). *In situ* hybridization of radiolabeled probes for most of these genes to metaphase cells from bone marrow aspirates of several AML patients with a del(5q) revealed that, with the exception of the *CSF1R* gene, each of the genes examined was deleted in the 5q− chromosome (19).

Based on their biological activity, several of these genes are good candidates for a tumor-suppressor gene. Alternatively, they may contribute to gene dosage effects. For this reason, we used genomic clones for these loci for FISH analysis to refine their localizations and to determine the order of these genes by dual-color FISH (Fig. 2*A*, Table 2). In addition, we used dual-color FISH to determine the relationship of these genes to the nine cosmid clones in 5q31 (Fig. 2*B*, Table 2). Our results indicate that the *IL3/CSF2* and *IL4/IL5/IRF1* gene clusters are proximal to *TCF7*; however, we were unable to determine the relative order of the two clusters, as the probes were too close to resolve the signals on metaphase cells. *EGR1* and *IL9* are proximal to *D5S166*. *CD14* is between *D5S166* and *D5S147*, whereas *FGFA* is between *D5S147* and *D5S178*. *SPARC* is distal to the cosmids. Finally, *ADRA1* is within 5q33, a band that is slightly distal to its previous localization (5q31–32) (16). This order is consistent with that obtained by Warrington *et al.* (21).

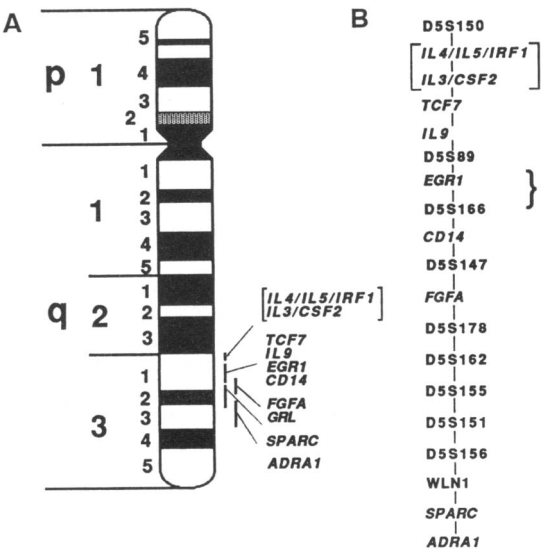

FIG. 2. Schematic diagram of the banding pattern of chromosome 5 illustrating the chromosomal localization and order of the *IL4/IL5/IRF1*, *IL3/CSF2*, *TCF7*, *IL9*, *EGR1*, *CD14*, *FGFA*, *GRL*, *SPARC*, and *ADRA1* genes determined by FISH (*A*) and the physical order of cosmid and phage clones and genes and the relationship of loci on 5q to the critical region of 5q31 (brace) (*B*). Brackets identify probes for which the order is unknown.

Molecular Delineation of the Critical Region. To identify probes that were located within the commonly deleted segment of 5q31, and to delineate the critical region at the molecular level, we performed FISH of the cosmid and gene

Table 2. Dual-color FISH analysis of genes and anonymous probes on 5q

Probe 1	Probe 2	No. of cells (chromosomes) analyzed	Probe 1 proximal, no. (%)	Probe 1 distal, no. (%)	Signals even, no. (%)
*IL4/IL5**	*IL3/CSF2*	17 (27)	6 (22)	13 (48)	8 (30)
IL4/IL5	*TCF7*	25 (31)	27 (87)	2 (6.5)	2 (6.5)
TCF7	*IL9*	35 (48)	36 (75)	6 (12.5)	6 (12.5)
IL9	*EGR1*	25 (41)	31 (76)	4 (10)	6 (15)
EGR1	*CD14*	18 (30)	25 (83)	0	5 (17)
CD14	*FGFA*	30 (43)	35 (82)	4 (9)	4 (9)
FGFA	*GRL*	45 (53)	38 (72)	15 (28)	0
GRL	*SPARC*	21 (31)	31 (100)	0	0
SPARC	*ADRA1*	10 (14)	11 (79)	2 (14)	1 (7)
D5S150†	*IL4*	10 (20)	17 (85)	2 (10)	1 (5)
TCF7	*D5S89*	20 (37)	24 (65)	6 (16)	7 (19)
D5S89	*EGR1*	39 (50)	32 (64)	14 (28)	4 (8)
EGR1	*D5S166*	20 (33)	29 (88)	0	4 (12)
D5S166	*CD14*	35 (45)	32 (71)	4 (9)	9 (20)
CD14	*D5S147*	29 (42)	27 (64)	11 (26)	4 (10)
D5S147	*FGFA*	16 (20)	24 (89)	3 (11)	0
FGFA	*D5S178*	25 (31)	20 (65)	4 (13)	7 (23)
WLN1	*SPARC*	10 (19)	17 (89)	2 (11)	0

Hybridization of many other probe combinations was used to confirm probe order; only those probe combinations that establish order are shown here.

*Order could not be determined by analysis of metaphase cells.

†FISH analysis of a cell line with a constitutional deletion of 5q, del(5)(q23.3q31.1), confirmed this order; *D5S150* was found to be proximal to *IL3/CSF2* and *IL4/IL5*.

Table 3. FISH analysis of leukemia cells with a del(5q)

	Distal breakpoint within 5q31							Proximal breakpoint within 5q31								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14*	15	16	17
	t-AML	t-MDS	AML	RAEB	RA	t-AML	t-AML	AML	AML	AML-M1	AML-M1	AML	AML-M5	ALL	AML-M1	t-MDS	MDS
	q13	q12	q13	q21	q15	q13	q15	q31	q31	q31	q31	q31	q3?1	q31	q31	q31	q31
Probe	q31	q31	q31	q31	q31	q31	q31	q33	q35	q35	q33	q33	q3?5	q33	q33	q33	q33or34
cen																	
D5S150		−						+	+	−						+	−
Il4/IL5		−						+	+	−							
IL3/CSF2		−						+	+	−							
TCF7	−		−	−	−	−	−	+	+	−	−	−		−	−	−	−
IL9									+	−	−				−	−	−
EGR1†	−		−	−	−	−		−	−	−				−	−	−	−
D5S166	−	−	−	−	+	+		−	−	−	−	−	−	−	−	−	−
D5S147	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−
D5S178		−	−	+		+	−	−	−	−					−	−	
D5S162		−	−					−	−	−		−	−	−	−	−	
D5S155		−	−					−	−	−							
D5S151		−						−	−	−							
D5S156	−	−	+					−	−	−							
WLN1	−	−	+		+		+	−	−	−							
92.15			+														
tel																	

Cells from 17 patients were analyzed. Disease and proximal and distal breakpoints are given below each patient number. Hybridization results are indicated (−, no signal; +, signal). ALL, acute lymphoblastic leukemia; RAEB, RA with excess blasts; cen, centromere; tel, telomere.
*A del(5q) was observed in the leukemia cells of this patient at the time of relapse following bone marrow transplantation. The analysis of the del(5q) in 135 patients illustrated in Fig. 1 is limited to patients with MDS or AML; thus, patient 14 is not included in this analysis.
†Only the *EGR1* probe gave no signal on the deleted homolog in each patient examined.

probes within 5q31 to metaphase cells from 17 patients who had either a proximal breakpoint (10 patients) or distal breakpoint (7 patients) within 5q31 (Table 3). The results reveal that *CSF2/IL3*, *IL4/IL5/IRF1*, *TCF7*, and *IL9* are proximal to the critical region and are not deleted in all patients, whereas *D5S166* and all cosmids telomeric to this marker are distal to the critical region (these markers are not deleted in all patients). Only *EGR1* is deleted in all patients (Table 3). In all cases, hybridization of the *EGR1* probe was observed on the cytogenetically normal homolog, suggesting that homozygous deletion of *EGR1* had not occurred.

These results are important because they identify proximal and distal markers that flank the critical region as well as a marker within this region. The flanking markers, *IL9* on the proximal side and *D5S166* on the distal side, were not included in the deleted segment; thus, these results suggest that a leukemia-suppressor gene is located between these loci. By multipoint genetic linkage analysis, we determined previously that the genetic distance between *D5S150* and *D5S166* is 6.7 cM (C.A.W., M.M.L.B., W.L.N., M. Mullan, and R. Williamson, unpublished data). We used PFGE to prepare a preliminary physical map of this genetic interval; the estimated minimum size is 4.7 Mb (Fig. 3). Two groups of genes are linked physically within this region (*IL4/IL5/IRF1* and *IL3/CSF2*) (17), but we were unable to link *TCF7*, *D5S89*, and *EGR1* to any other markers. The minimum

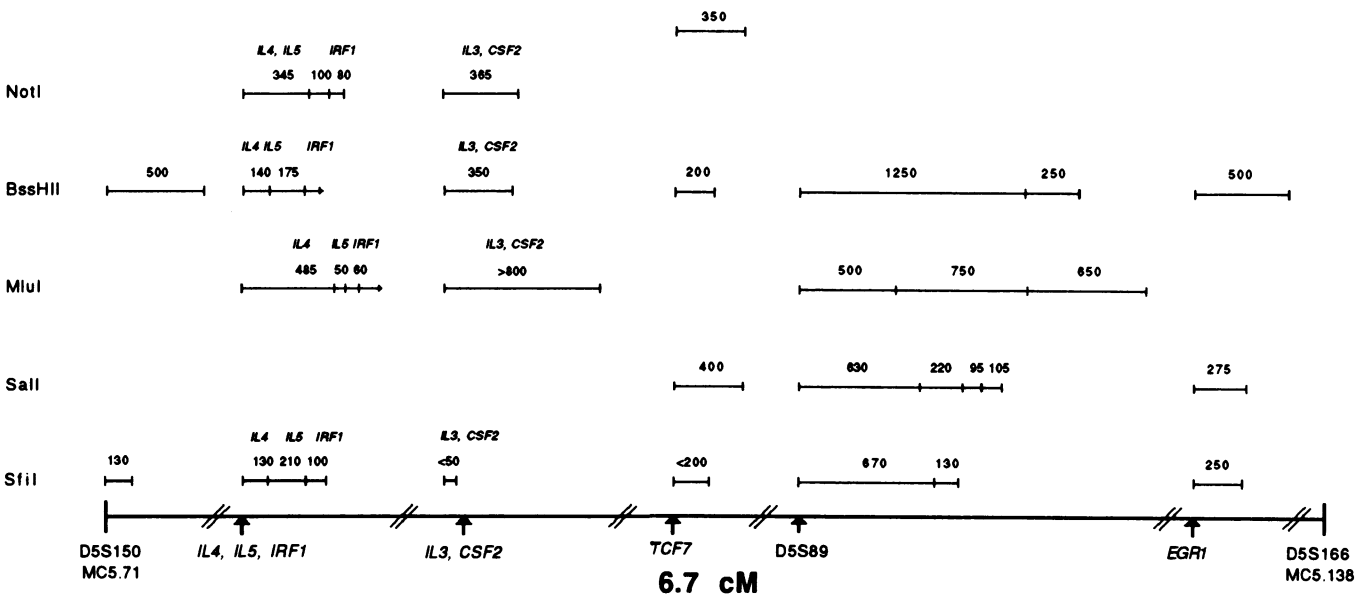

FIG. 3. Physical map of the genetic interval between *D5S150* and *D5S166*. Restriction endonucleases used to digest human DNA are shown on the vertical axis. Markers mapped by PFGE are on the horizontal axis. Fragment sizes, in kilobases, are shown above the horizontal bars. Double diagonal lines indicate that adjacent markers are not linked by PFGE. The genetic length of this interval is 6.7 cM.

physical distance between *TCF7* and *D5S166* is estimated to be 2.8 Mb (Fig. 3).

Analysis of the *EGR1* Gene in t-MDS/t-AML Patients. To determine whether molecular deletions encompassing *EGR1* occurred in myeloid leukemia cells in the absence of detectable cytogenetic abnormalities leading to loss of 5q, we performed FISH analysis of bone marrow cells from nine patients with t-MDS/t-AML, using the *EGR1* probe. Five patients had loss or deletion of chromosome 7, three patients had abnormalities involving chromosomes other than 5 or 7, and one patient had a normal karyotype. In each patient, we detected signal at band q31 of both chromosome 5 homologs, including those homologs examined from the abnormal clonal cells in eight of the patients. These results suggested that *EGR1* is not deleted as a result of submicroscopic molecular deletions in patients with t-AML who do not have abnormalities of chromosome 5. Whether subtle mutations of *EGR1* have occurred is unknown.

DISCUSSION

Our results of cytogenetic and molecular mapping of the deletions of chromosome 5 in myeloid disorders suggest that the gene(s) that is involved in the pathogenesis of these disorders is likely to be located in 5q31 between the *IL9* and *D5S166* loci. Several experimental approaches, such as candidate gene and physical mapping approaches, can be used to identify a putative tumor-suppressor gene. In the first approach, one seeks to identify mutations of a gene(s) that is known to map to the critical region in tumor cells, whereas the second approach involves the translation of the cytogenetic map of the critical region into a physical map, in which the deleted regions are identified by markers with defined physical and genetic locations. Such a map could be used to screen DNA from leukemia cells with abnormalities of chromosome 5 to identify alterations of genomic sequences within the critical region. The preparation of a cytogenetic map of 5q31 and the identification of probes within and flanking the critical region represent key steps in identifying a myeloid tumor-suppressor gene on 5q.

The identification of a cluster of genes encoding hematopoietic growth factors in 5q31 suggests a role for these genes in the autocrine growth of myeloid leukemia cells. However, autocrine growth of leukemia cells with a del(5q) as a result of aberrant expression of a growth factor gene located on 5q has not been documented. Further, our results indicate that these genes are proximal to the commonly deleted segment of 5q31. Nonetheless, we cannot exclude the possibility that hemizygous deletion of these genes, which would occur in most patients with a del(5q), may result in a gene dosage effect and, thereby, contribute to the phenotype associated with these neoplastic diseases.

We demonstrated that the *EGR1* gene is located within the commonly deleted segment of chromosome 5. The properties of *EGR1* and its encoded protein make this gene a suitable candidate for a tumor-suppressor gene (22). The EGR1 protein is a DNA-binding zinc-finger protein with transcriptional regulatory activity; *EGR1* is upregulated during terminal myeloid differentiation (V. P. Sukhatme, personal communication) and is essential for and restricts differentiation of myeloblasts along the macrophage lineage (23). EGR1 protein binds to the same target DNA sequence as does the product of the *WT1* gene, a candidate tumor-suppressor gene in Wilms tumor. The status of *EGR1* in myeloid leukemia cells is unknown; however, Gilliland *et al.* (24) found no mutations of this gene by PCR analysis of clonally derived monocytes in 10 RA 5q− syndrome patients. Nonetheless, a more detailed molecular characterization of *EGR1* in myeloid leukemia cells is warranted. Alternatively, the deletions of 5q may result in a gene dosage effect. This mechanism would be compatible with the results obtained by Gilliland *et al.* (24) described above. In this event, molecular mapping of the breakpoint in patients with AML who have balanced translocations involving 5q31 may pinpoint the location of the involved gene.

More recently, Willman *et al.* (25) have detected a rearrangement of the *IRF1* gene in 1 patient with acute lymphoblastic leukemia and heterozygous deletions of this gene in 12 other leukemia patients. The results of our studies suggest that *IRF1* is proximal to the commonly deleted segment. Thus, it is possible that the distal 5q contains more than one gene that is involved in myeloid leukemogenesis.

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1. Mitelman, F., Kaneko, Y. & Trent, J. (1991) *Cytogenet. Cell Genet.* **58,** 1053–1079.
2. Klein, G. (1988) *Science* **238,** 1539–1545.
3. Seizinger, B. R., Klinger, H. P., Junien, C., Nakamura, Y., Le Beau, M. M., Cavenee, W., Emanuel, B., Ponder, B., Naylor, S., Mitelman, F., Louis, D., Menon, A., Newsham, I., Decker, J., Kaelbling, M., Henry, I. & von Deimling, A. (1992) *Cytogenet. Cell Genet.* **58,** 1080–1096.
4. Koeffler, H. P. & Rowley, J. D. (1985) in *Neoplastic Diseases of the Blood,* eds. Wiernik, P. H., Canellos, G. P., Kyle, K. A. & Schiffer, C. A. (Churchill Livingstone, New York), Vol. 1, pp. 357–381.
5. Rowley, J. D., Golomb, H. M. & Vardiman, J. W. (1981) *Blood* **58,** 759–767.
6. Pedersen-Bjergaard, J., Philip, P., Larsen, S. O., Jensen, G. & Byrsting, K. (1990) *Blood* **76,** 1083–1091.
7. Le Beau, M. M., Albain, K. S., Larson, R. A., Vardiman, J. W., Davis, E. M., Blough, R. R., Golomb, H. M. & Rowley, J. D. (1986) *J. Clin. Oncol.* **4,** 325–345.
8. Nimer, S. D. & Golde, D. W. (1987) *Blood* **70,** 1705–1712.
9. Van den Berghe, H., Vermaelen, C., Mecucci, C., Barbieri, D. & Tricot, G. (1985) *Cancer Genet. Cytogenet.* **17,** 189–255.
10. Bennett, J. M., Catovsky, D., Daniel, M. T., Flandrin, G., Galton, D. A. G., Gralnick, H. R. & Sultan, C. (1982) *Br. J. Haematol.* **51,** 189–199.
11. Bennett, J. M., Catovsky, D., Daniel, M. T., Flandrin, G., Galton, D. A. G., Gralnick, H. R. & Sultan, C. (1985) *Ann. Intern. Med.* **103,** 626–629.
12. Nakamura, Y., Lathrop, M., Leppert, M., Dobbs, M., Wasmuth, J., Wolff, E., Carlson, M., Fujimoto, E., Krapcho, K., Sears, T., Woodward, S., Hughes, J., Burt, R., Gardner, E., Lalouel, J.-M. & White, R. (1988) *Am. J. Hum. Genet.* **43,** 638–644.
13. Williamson, R., Bowcock, A., Kidd, K., Pearson, P., Schmidtke, J., Cerverha, P., Chipperfield, M., Cooper, D. N., Coutelle, C., Hewitt, J., Klinger, K., Langley, K., Beckmann, J., Tolley, M. & Maidak, B. (1991) *Cytogenet. Cell Genet.* **58,** 1190–1832.
14. Neuman, W. L., Westbrook, C. A., Dixon, M., Espinosa, R., III, Patel, Y. D., Nakamura, Y., Weiffenbach, B. & Le Beau, M. M. (1993) *Cytogenet. Cell Genet.* **62,** 207–210.
15. Neuman, W. L., Le Beau, M. M., Farber, R. A., Lindgren, V. & Westbrook, C. A. (1992) *Cytogenet. Cell Genet.* **61,** 103–106.
16. Wasmuth, J. J., Bishop, D. T. & Westbrook, C. A. (1991) *Cytogenet. Cell Genet.* **58,** 261–294.
17. Stock, W., Chandrasekharappa, S. C., Neuman, W. L., Le Beau, M. M., Brownstein, B. H. & Westbrook, C. A. (1992) *Cytogenet. Cell Genet.* **61,** 263–265.
18. Rowley, J. D., Diaz, M. O., Espinosa, R., Patel, Y. D., van Melle, E., Ziemin, S., Taillon-Miller, P., Lichter, P., Evans, G. A., Kersey, J. D., Ward, D. C., Domer, P. H. & Le Beau, M. M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 9358–9362.
19. Le Beau, M. M., Chandrasekharappa, S. C., Lemons, R. S., Schwartz, J. L., Larson, R. A., Arai, N. & Westbrook, C. A. (1989) *Cancer Cells* **7,** 53–58.
20. Trask, B. J., Massa, H., Kenrick, S. & Gitschier, J. (1991) *Am. J. Hum. Genet.* **48,** 1–15.
21. Warrington, J. A., Bailey, S. K., Armstrong, E., Aprelikova, O., Alitalo, K., Dolganov, G. M., Wilcox, A. S., Sikela, J. M., Wolfe, S. F., Lovett, M. & Wasmuth, J. J. (1992) *Genomics* **13,** 803–808.
22. Sukhatme, V. P., Cao, X., Chang, L. C., Tsai-Morris, C.-H., Stamenkovich, D., Ferreira, P. C. P., Cohen, D. R., Edwards, S. A., Shows, T. B., Curran, T., Le Beau, M. M. & Adamson, E. D. (1988) *Cell* **53,** 37–43.
23. Nguyen, H. Q., Hoffman-Liebermann, B. & Liebermann, D. A. (1993) *Cell* **72,** 197–209.
24. Gilliland, D. G., Perrin, S. & Bunn, H. F. (1991) *Leukemia Res.* **15,** Suppl., PC21.
25. Willman, C., Sever, C., Pallavicini, M., Harada, H., Tanaka, N., Slovak, M., Yamamoto, H., Harada, K., List, A. & Taniguchi, T. (1993) *Science* **259,** 968–971.