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Assignment of *CSF-1* to 5q33.1: Evidence for clustering of genes regulating hematopoiesis and for their involvement in the deletion of the long arm of chromosome 5 in myeloid disorders

(gene mapping/hematopoietic growth factors/myeloid leukemias/chromosomal deletions/malignant transformation)

MARK J. PETTENATI*, MICHELLE M. LE BEAU*§, RICHARD S. LEMONS†, ELIZABETH A. SHIMA*, ERNEST S. KAWASAKI‡, RICHARD A. LARSON*, CHARLES J. SHERR†, MANUEL O. DIAZ*, AND JANET D. ROWLEY*

*Joint Section of Hematology/Oncology, Department of Medicine, Box 420, University of Chicago, Chicago, IL 60637; †Department of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN 38101; and ‡Department of Molecular Biology, Cetus Corp., 1400 53rd Street, Emeryville, CA 94608

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ABSTRACT **The *CSF-1* gene encodes a hematopoietic colony-stimulating factor (CSF) that promotes growth, differentiation, and survival of mononuclear phagocytes. By using somatic cell hybrids and *in situ* hybridization, we localized this gene to human chromosome 5 at bands q31 to q35, a chromosomal region that is frequently deleted [del(5q)] in patients with myeloid disorders. By *in situ* hybridization, the *CSF-1* gene was found to be deleted in the 5q− chromosome of a patient with refractory anemia who had a del(5)(q15q33.3) and in that of a second patient with acute nonlymphocytic leukemia *de novo* who had a similar distal breakpoint [del(5)(q13q33.3)]. The gene was present in the deleted chromosome of a third patient, with therapy-related acute nonlymphocytic leukemia, who had a more proximal breakpoint in band q33 [del(5)(q22q33.1)]. Hybridization of the *CSF-1* probe to metaphase cells of a fourth patient, with acute nonlymphocytic leukemia *de novo*, who had a rearrangement of chromosomes 5 and 21 [ins(21;5)(q22;q31.3q33.1)] resulted in labeling of the breakpoint junctions of both rearranged chromosomes; this suggested that *CSF-1* is located at 5q33.1. Thus, a small segment of chromosome 5 contains *GM-CSF* (the gene encoding the granulocyte-macrophage CSF), *CSF-1*, and *FMS*, which encodes the CSF-1 receptor, in that order from the centromere; this cluster of genes may be involved in the altered hematopoiesis associated with a deletion of 5q.**

Loss of a whole chromosome 5 or loss of a part (deletion) of the long arm of this chromosome [del(5q)] is frequently observed in the malignant cells of patients with a myelodysplastic syndrome (MDS) or those with acute nonlymphocytic leukemia (ANLL) secondary to cytotoxic therapy for a previous malignant disease [therapy-related MDS or ANLL (t-MDS, t-ANLL)] (1–5). It is also observed less frequently in patients with ANLL arising *de novo* (6–9). The relatively high frequency of loss of chromosome 5 or del(5q) in patients with t-ANLL and the relative absence of these abnormalities in the leukemia cells of patients with ANLL *de novo* who are <30 years old have led to the suggestion that these abnormalities may be a marker of mutagen-induced leukemia (10). A del(5q) also occurs in patients with a primary MDS characterized by refractory anemia (RA) with abnormal megakaryocytes (11, 12); the disorder in this latter group of patients has been termed the "5q− syndrome."

The deletions of chromosome 5 observed in the RA 5q− syndrome and in ANLL are interstitial rather than terminal and are characterized by variability in the proximal and distal breakpoints. However, in a recent clinical and cytogenetic evaluation of t-ANLL and t-MDS patients, a region was identified consisting of bands 5q23–32, which was deleted in all patients. This segment has been termed the critical region (5). The identification of a critical region suggests that the loss of genes located within this region plays an important role in the pathogenesis of these hematologic disorders (5, 10).

Recently, the gene encoding the granulocyte-macrophage colony-stimulating factor (*GM-CSF*) and the protooncogene *FMS* have been localized within or adjacent to the critical region of chromosome 5 (13). Colony-stimulating factors (CSFs) are a family of glycoproteins that are believed to be required for growth and maturation of myeloid progenitor cells *in vivo* and *in vitro* (14, 15). In cell culture systems, murine multi-CSF (interleukin 3, IL-3) stimulates the progenitor cells of most of the hematopoietic cell lineages (16), whereas GM-CSF stimulates the proliferation of cells from the granulocyte, granulocyte-macrophage, and macrophage lineages (17). Macrophage-CSF (M-CSF or CSF-1) (18) and granulocyte-CSF (G-CSF) (19) primarily stimulate cells committed to the macrophage and granulocyte lineages, respectively. In addition to their capacity to stimulate the proliferation of progenitor cells, the CSFs can induce commitment to differentiate in these precursors and can stimulate the functional activity of mature granulocytes and macrophages (14).

In humans, these factors are less well characterized; however, human GM-CSF, CSF-1, and G-CSF have recently been purified, and cDNA clones have been isolated (20–23). Genomic sequences encoding human GM-CSF have also been cloned, and this gene has been mapped to human chromosome 5, at bands q23–31 (13, 24). The *FMS* protooncogene, localized to 5q33.2–33.3 (13), encodes a protein with an associated tyrosine kinase activity that is closely related, and possibly identical, to the receptor for CSF-1 (25). Thus, *FMS* also plays a role in the differentiation and proliferation of mononuclear phagocytic cells (25, 26). *In situ* hybridization studies previously suggested that the deletion of *FMS* and *GM-CSF* in the 5q− syndrome or in ANLL may result in hemizygosity for critical loci, thereby leading to abnormal hematopoiesis (13, 26). Using a probe derived from the recently cloned *CSF-1* gene (21) for *in situ* chromosomal hybridization and somatic cell hybrid analysis, we have

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Abbreviations: ANLL, acute nonlymphocytic leukemia; CSF, colony-stimulating factor; *CSF-1*, gene encoding the macrophage colony-stimulating factor; del(5q), deletion of the long arm of chromosome 5; *FMS*, cellular homologue of the transforming gene of McDonough feline sarcoma virus, formerly c-*fms*; *GM-CSF*, gene encoding the granulocyte-macrophage colony-stimulating factor; RA, refractory anemia; t-ANLL, therapy-related ANLL; MDS, myelodysplastic syndrome; t-MDS, therapy-related MDS; G-CSF, granulocyte-CSF.

§To whom reprint requests should be addressed.

demonstrated that the *CSF-1* gene is closely linked to *FMS* on human chromosome 5 and that it is located adjacent to the critical region of this chromosome.

MATERIALS AND METHODS

Patients. The clinical and cytogenetic features of the four patients with hematologic diseases characterized by a deletion of chromosome 5, or by a balanced rearrangement leading to a loss of material from chromosome 5, are listed in Table 1. One patient had the RA 5q− syndrome, two patients had ANLL arising *de novo*, and one patient had t-ANLL following chemotherapy and radiotherapy for Hodgkin disease. Three patients had a del(5q); of these, patient 1 had a deletion extending from bands q15 to q33.3, patient 2 had a larger deletion extending from bands q13 to q33.3, and patient 3 had a distal breakpoint that was more proximal within band q33 [del(5)(q22q33.1)]. The remaining patient (4) had a rearrangement involving chromosomes 5 and 21 in which bands 5q31.3 to q33.1 are inserted into chromosome 21 at band q22 [ins(21;5)]. Thus, metaphase cells from this patient are particularly useful for the sublocalization of genes within bands q31, q32, and q33 of chromosome 5.

DNA Probes. The CSF-1 probe used is a 900-base pair (bp) *Bam*HI fragment from a *CSF-1* cDNA clone and contains bp 512 to the 3′ end of the mRNA (21). The FMS probe is a 3.3-kilobase (kb) *Eco*RI genomic fragment containing 5′ intron sequences of the human *FMS* gene cloned in pUC9 (26, 27).

***In Situ* Chromosomal Hybridization.** A radiolabeled CSF-1 probe was prepared by nick-translation of the entire plasmid with all four ^{3}H-labeled deoxynucleoside triphosphates to a specific activity of 1.8×10^8 dpm per μg. *In situ* hybridization was performed as previously described (28). Metaphase cells were hybridized at 4.0 and 8.0 ng of probe per ml of hybridization mixture. Autoradiographs were exposed for 11 days.

Somatic Cell Hybrids. Somatic cell hybrids were formed by polyethylene glycol-mediated fusion of human VA2 (gift of D. H. Poon, National Institutes of Health), A549 (American Type Culture Collection CCL 185), and IMR-90 fibroblast cells [National Institute of General Medical Sciences (NIGMS) Human Genetic Mutant Cell Repository AG3204] to Chinese E36 (gift of C. T. Caskey, Baylor University), or Syrian BHK-B1 hamster cells (NIGMS HGMCR GM0345) that were mutant in their hypoxanthine-phosphoribosyltransferase or thymidine kinase genes, respectively, permitting hybrid selection with hypoxanthine/aminopterin/thymidine (HAT) medium. Since human cells cannot survive in 10 μM ouabain, hybrids were initially grown in HAT/ouabain medium. A panel for mapping studies was established from a series of hybrids that contain the entire rodent genome but have selectively lost different combinations of human chromosomes. Their human chromosome content was determined by screening for up to 34 gene enzyme systems (29) and, in selected cases, by cytogenetic analyses using trypsin-Giemsa banding (30). High molecular weight DNA for Southern blots and cell homogenates for isozymes were prepared from the same passages of cells.

The CSF-1 probe detects a single *Hin*dIII or two *Eco*RI restriction fragments in human DNA that are distinct from those in hamster DNA. This clone was ^{32}P-radiolabeled by use of random oligonucleotide primers and Klenow DNA polymerase (31) to a specific activity of $1\text{–}4 \times 10^8$ dpm per μg of DNA and was annealed to Southern blots of *Hin*dIII- or *Eco*RI-digested DNAs from the hybrid cell panel. The Southern blots were washed and rehybridized with the FMS probe, which detects a single *Eco*RI, *Hin*dIII, or *Bam*HI restriction fragment in human DNA, distinct from hamster DNA (27).

Gel Electrophoresis, Southern Transfer, and Hybridization of DNA. Southern blot analysis of DNA from leukemia cells with abnormalities of chromosome 5 was performed as described previously (32).

Table 2. Synteny test of the *CSF-1* gene and human chromosomes in rodent–human hybrid clones

Human chromosome	*CSF-1* gene/human chromosome				
	+/+	+/−	−/+	−/−	% asynteny
1	6	11	5	7	55
2	5	12	3	11	48
3	2	10	0	0	83
4	0	12	0	0	100
5	17	1*	0	13	3
6	9	6	7	6	46
7	0	12	0	0	100
8	3	15	3	9	60
9	13	3	2	5	22
10	5	12	3	3	65
11	9	8	6	2	56
12	10	9	5	9	42
13	4	8	0	0	67
14	13	4	7	7	35
15	9	8	8	5	53
16	15	2	4	4	24
17	11	1	16	0	61
18	3	13	3	3	73
19	4	12	7	3	73
20	9	8	6	4	52
21	1	11	0	0	92
22	0	12	0	0	100
X	11	6	10	4	52

*This clone lacks human HEXB activity and *FMS* coding sequences, but contains *CSF-1* coding sequences as determined by Southern blot hybridization.

RESULTS

Analysis of Somatic Cell Hybrids. To determine the chromosomal location of *CSF-1*, we established a panel from a series of somatic cell hybrids that contain the entire rodent genome, but have selectively lost different combinations of human chromosomes. Southern blot analyses of this hybrid panel showed concordance between the presence of human *CSF-1* sequences and human chromosome 5 (Table 2). As an internal standard for confirmation of this assignment, the Southern blots were washed and hybridized with the FMS probe previously assigned to chromosome 5 (27). Of 31 clones examined, only one showed a discordance between the presence of human chromosome 5 and *FMS* and *CSF-1* sequences. This clone contained human *CSF-1* sequences, but it lacked *FMS* sequences and human hexosaminidase-B

Table 1. Clinical and cytogenetic features of patients with a del(5q) or ins(21;5)

Patient	Age/sex	Hematologic disease	Karyotype
1	61/M	RA	46,XY(45%)/46,XY,del(5)(q15q33.3)(55%)
2	63/M	AML	46,XY,del(5)(q13q33.3),t(19;?)(p13;?)(95%)/46,XY,del(5)(5%)
3	28/M	t-ANLL	46,XY(10%)/45,XY,−7(7%)/45,XY,−7,del(5)(q22q33.1)(83%)
4	59/F	AMMoL	46,XX(27%)/47,XX,−7,+8,+der(1)t(1;7)(p11;p11),ins(21;5)(q22;q31.3q33.1)(73%)

AML, acute myeloblastic leukemia; AMMoL, acute myelomonocytic leukemia.

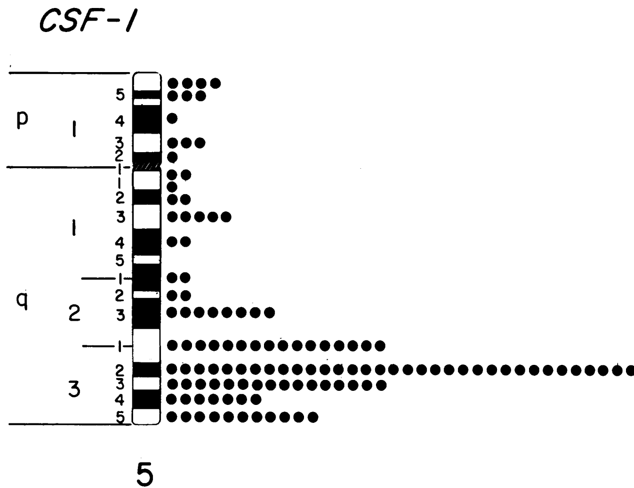

FIG. 1. Distribution of labeled sites on chromosome 5 in 200 normal metaphase cells from phytohemagglutinin-stimulated peripheral blood lymphocytes that were hybridized with a ^{3}H-labeled *CSF-1*-specific probe. Each dot represents one labeled site observed in the corresponding band. Sixty-eight percent (84/120) of the labeled sites on chromosome 5 were located at bands q31–35; this cluster represented 23% of all labeled sites (84/374).

activity, and it had no cytogenetically identifiable human chromosome 5.

***In Situ* Chromosomal Hybridization.** To determine the chromosomal sublocalization of the *CSF-1* gene, we hybridized the CSF-1 probe to normal human metaphase chromosomes. This resulted in specific labeling only of chromosome 5. Of 200 metaphase cells examined, 80 (40%) were labeled on region q3 of one or both chromosomes 5. The distribution of labeled sites on this chromosome is illustrated in Fig. 1; of 374 labeled sites observed, 120 (32%) were located on this chromosome. These sites were clustered on bands q31 to q35, and this cluster represented 23% (84/374) of all labeled sites ($P < 0.0005$).

To determine the relationship of *CSF-1* to the critical region of chromosome 5, we hybridized the CSF-1 probe to metaphase cells obtained from bone marrow aspirates of the three patients described earlier with the RA 5q− syndrome, ANLL *de novo*, or t-ANLL characterized by a del(5q). The results of these hybridizations are listed in Table 3 and are illustrated in Fig. 2. We have previously reported *in situ* hybridization data for *GM-CSF* and *FMS* on patients 1 and 3 (13). In all three cases, specific labeling was observed on the normal chromosome 5 homologues at bands q31–35 (Fig. 2 *Left* and Table 3). Grain accumulation was not observed on the deleted homologues in patients 1 and 2 (Fig. 2 *Right* and Table 3). Hence, the *CSF-1* locus was deleted in patients who

FIG. 2. Distribution of labeled sites on the normal chromosome 5 and on the deleted homologues in metaphase cells from bone marrow aspirates from patients 1, 2, and 3 that were hybridized with the CSF-1 probe. The arrows to the left of the normal chromosome 5 (*Left*) illustrate the breakpoints and thus the segment that is deleted in each patient. The arrow on each 5q− chromosome (*Right*) identifies the breakpoint junction. The results of these hybridizations indicate that *CSF-1* is deleted in patients 1 and 2, in whom the distal breakpoint is in band q33.3, but not in patient 3, who has a more proximal breakpoint within this band (q33.1).

had a distal breakpoint at band q33.3. In contrast, *CSF-1* was present in the deleted chromosome 5 of patient 3, whose distal breakpoint had been determined to be 5q33.1 (Fig. 2 *Right* and Table 3). This suggested that *CSF-1* is located distal to or at band q33.1, but proximal to band q33.3.

We further sublocalized *CSF-1* by hybridization of this probe to the leukemia metaphase cells of patient 4, who had ANLL *de novo* and a rearranged chromosome 5 in which

Table 3. *In situ* hybridization of the human CSF-1 probe to leukemia metaphase cells from three patients with a del(5q) and one patient with an ins(21;5)

			Labeled sites, %					
			Normal chromosome 5		5q− chromosome			
Patient	Cells analyzed, no.	Labeled sites, no.	Total	Bands q31–35	Total	Bands q23–35	Normal chromosome 21, Total	21q+ chromosome, Total
1	45	106	35 (33.0%)*	25 (23.6%)	3 (2.8%)	2 (1.9%)†		
2	188	445	70 (15.5%)*	59 (13.3%)	8 (1.8%)	2 (0.5%)‡		
3	106	283	52 (18.4%)*	44 (15.6%)	35 (12.4%)*	32 (11.3%)§		
4	165	420	69 (16.5%)*	55 (13.1%)	49 (11.7%)*	41 (9.8%)¶	4 (1%)	43 (10.2%)*¶

*χ^2 value corresponds to a $P < 0.0005$; the χ^2 statistic tests the hypothesis that labeling is random on all chromosomes.
†Bands q15 to q33.3 are deleted.
‡Bands q13 to q33.3 are deleted.
§Bands q22 to q33.1 are deleted.
¶Bands 5q31.3, 5q32, and 5q33.1 are inserted into chromosome 21 at band q22.

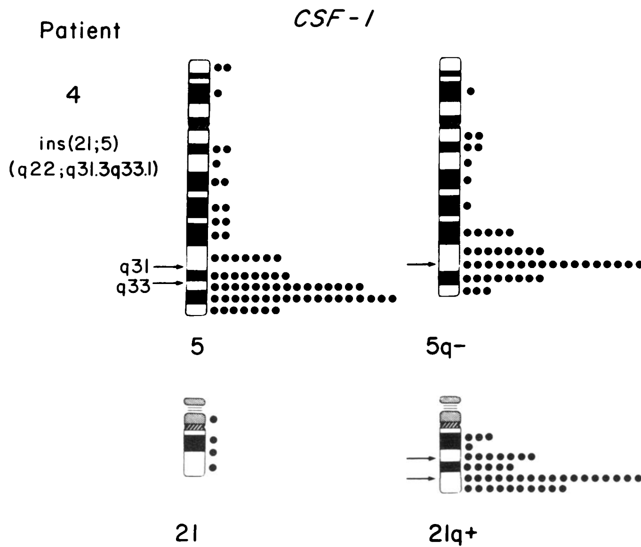

FIG. 3. Distribution of labeled sites on the normal chromosomes 5 and 21 and on the rearranged homologues in metaphase cells from patient 4 with an ins(21;5) that were hybridized with the CSF-1 probe. In this chromosomal rearrangement, bands 5q31.3 to q33.1 are inserted into chromosome 21 at band q22. The arrows on the normal chromosome 5 (*Upper Left*) identify the breakpoints and the segment that is inserted into chromosome 21 (*Lower Right*, arrows). The arrow on the 5q− chromosome (*Upper Right*) identifies the breakpoint junction; this hybridization labeled the 5q− and the 21q+ chromosomes, thus sublocalizing *CSF-1* to band q33.1.

bands 5q31.3 to 5q33.1 were inserted into chromosome 21 at band q22 [ins(21;5)(q22;q31.3q33.1)]. Metaphase cells from this patient had previously proved useful in the sublocalization of *FMS* to band q33.2–33.3 (13). Labeling was observed both on the long arm of the rearranged chromosome 5 and on the rearranged chromosome 21 (Fig. 3 and Table 3), suggesting that *CSF-1* is located at band q33.1 and is proximal to *FMS*. Although these data suggest that this particular chromosomal rearrangement splits the *CSF-1* gene, we cannot exclude the possibility that the *CSF-1* sequences were duplicated on the two rearranged chromosomes. These results, together with those of our earlier studies (13), suggest that the order of the *CSF* and *FMS* genes on the long arm of chromosome 5 is: centromere, *GM-CSF, CSF-1, FMS*, telomere.

Southern Blot Analysis. To determine whether the *CSF-1* gene was rearranged as a consequence of the chromosomal rearrangement in patient 4 [ins(21;5)], we hybridized Southern blots of DNA that was extracted from leukemia cells of this patient and digested with various restriction enzymes to the CSF-1 probe. Only germline *Hin*dIII, *Eco*RI, and *Bam*HI restriction fragments were observed (data not shown). These results are inconsistent with the occurrence of a break within the sequences encompassed by this probe. An alternative explanation for the findings by *in situ* hybridization is that a chromosomal segment containing the *CSF-1* gene was duplicated. Such a duplication might result from the rearrangement that gave rise to the ins(21;5). Alternatively, the *CSF-1* gene may be separated from an adjacent, partially homologous sequence as a result of the break at 5q33.1. The relatively low stringency conditions of *in situ* chromosomal hybridization may allow the detection of homologous sequences that are not identified by Southern blot hybridization.

DISCUSSION

By using *in situ* chromosomal hybridization and somatic cell hybrid analysis, we have localized the *CSF-1* gene to the long arm of chromosome 5 at band q33. In earlier studies we localized the *GM-CSF* and *FMS* genes to this region of chromosome 5, specifically to 5q23–31 and 5q33.2–33.3, respectively (13). The two *CSF* genes and the *FMS* gene are located in a region of chromosome 5 that is frequently deleted in patients with neoplastic myeloid disorders (1–13). Our results, together with previous studies of the *FMS* gene (26), showing that these genes are within the deleted segment of chromosome 5 in the RA 5q− syndrome and in ANLL suggest that they play some role in the pathogenesis of these disorders.

The relative variability of the breakpoints noted in the deletion of chromosome 5 suggests that the event which may be essential for malignant transformation is the loss of a critical DNA sequence, rather than the consistent juxtaposition of two genes as in the case of *ABL* and bcr (breakpoint cluster region) sequences in chronic myelogenous leukemia (33). The identification of a critical region—i.e., a chromosomal segment that is deleted in all patients with this rearrangement—further supports this hypothesis. Deletion of a *CSF* or *FMS* allele may result in a reduced level of the gene products. Another outcome of a deletion could be the loss of a wild-type gene, which allows the expression of a recessive mutant allele on the homologous chromosome. Alternatively, the loss of function of both alleles may have occurred, one through a detectable chromosomal deletion and the other as the result of a mutation. Similar mechanisms have been proposed for the pathogenesis of retinoblastoma and Wilms tumor (34, 35).

At present, it is not known whether the deletion of one (or several) gene encoding CSFs or the loss of the receptor gene(s) is the critical genetic alteration in malignant myeloid cells with a del(5q). With respect to the *CSF* genes, autocrine stimulation of cell proliferation is an intriguing phenomenon; however, there is little evidence to suggest that myeloid leukemia cells are proliferating as a result of an acquired capacity to synthesize their own CSFs. In fact, the proliferation of leukemia cells *in vitro*, like that of normal cells, is dependent on the addition of exogenous CSF, at concentrations similar to those required by normal cells (14). Some studies on leukemia monocytes have demonstrated, however, that they can produce CSF (14). Furthermore, experimental data suggesting that expression of *CSF* genes is important in malignant transformation were obtained recently. Lang *et al.* (36) showed that GM-CSF production induced by a retroviral expression vector resulted in CSF independence in a factor-dependent murine hematopoietic cell line. Moreover, these cells were leukemogenic when inoculated into mice, whereas the parental cell line was not.

On the other hand, loss of CSF receptor genes may be the relevant genetic alteration in these leukemia cells. The lack of capacity for autonomous growth of leukemia cells *in vitro* has led to the hypothesis that leukemia stem cells have an intrinsic abnormality such that CSF-stimulated proliferation causes an abnormally high ratio of self-regenerative divisions as compared with divisions leading to the production of differentiated cells (14). This defect could result from an altered level of expression of CSF receptor(s) or from the production of an altered receptor.

Our data provide evidence that a functional family of genes that regulate the growth of hematopoietic cells maps to a limited segment of chromosome 5, and they raise the possibility that genes encoding other growth factors and their receptors may be located within this region. The recent localization of the genes encoding the receptor for platelet-derived growth factor to 5q31–32 (37), the β_2-adrenergic receptor to 5q31–32 (38), and the endothelial cell growth factor to 5q31.3–32 (39) supports this hypothesis. The localization of *CSF-1* and *FMS* to the same chromosomal band (5q33) is particularly notable, because other growth factor

and growth factor receptor genes mapped to date are localized to different chromosomes—e.g., epidermal growth factor, platelet-derived growth factor, insulin, interleukin-2, and their receptors—or they have been mapped to different regions of the same chromosome, as is the case for transferrin and the transferrin receptor (40). The chromosomal location of a gene that appears to be the human counterpart to multi-CSF, or interleukin-3, has not yet been determined; however, the gene encoding the human granulocyte CSF (G-CSF) has recently been cloned (22, 23) and is located at 17q11–21 (M.M.L.B., R.S.L., and M.O.D., unpublished data; S. Nagata, personal communication). Genes encoding GM-CSF and interleukin-3 have both been localized to mouse chromosome 11 (14); this raises the possibility that these genes are adjacent. Finally, if many CSF and receptor genes prove to be located within this same region of chromosome 5, it may be possible to relate some of the clinical features of the somewhat heterogenous group of patients with a del(5q) to the specific chromosomal deletions, or to the possible alterations of the CSF or CSF receptor genes on the homologous chromosome 5.

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1. Rowley, J. D., Golomb, H. M. & Vardiman, J. W. (1981) *Blood* **58**, 759–767.
2. Sandberg, A. A., Abe, S., Kowalczyk, J. R., Zedgenidze, A., Takeuchi, J. & Kakati, S. (1983) *Cancer Genet. Cytogenet.* **7**, 95–136.
3. Pedersen-Bjergaard, J., Philip, P., Pederson, N. T., Hou-Jensen, K., Svejgaard, A., Jensen, G. & Nissen, N. I. (1984) *Cancer* **54**, 452–462.
4. Arthur, D. C. & Bloomfield, C. D. (1984) *Cancer Genet. Cytogenet.* **12**, 189–199.
5. 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.
6. Fourth International Workshop on Chromosomes in Leukemia 1982 (1984) *Cancer Genet. Cytogenet.* **11**, 296–299.
7. Mitelman, F. (1985) in *Progress and Topics in Cytogenetics*, ed. Sandberg, A. A. (Liss, New York), Vol. 5, pp. 107–137.
8. Van den Berghe, H., Vermaelen, K., Mecucci, C., Barbieri, P. & Tricot, G. (1985) *Cancer Genet. Cytogenet.* **17**, 189–255.
9. Dewald, G. W., Davis, M. P., Pierre, R. V., O'Fallon, J. R. & Hoagland, H. C. (1985) *Blood* **66**, 189–197.
10. Rowley, J. D. (1983) in *Chromosomes and Cancer: From Molecules to Man*, eds. Rowley, J. D. & Ultmann, J. E. (Academic, New York), pp. 139–159.
11. Van den Berghe, H., Cassiman, J. J., David, G. & Fryns, J. P. (1974) *Nature (London)* **251**, 437–438.
12. Teerenhovi, L., Borgström, G. H., Lintula, R., Ruutu, T., Lahtinen, R., de la Chapelle, A. & Vuopio, P. (1981) *Scand. J. Haematol.* **27**, 119–129.
13. Le Beau, M. M., Westbrook, C. A., Diaz, M. O., Larson, R. A., Rowley, J. D., Gasson, J. C., Golde, D. W. & Sherr, C. J. (1986) *Science* **231**, 984–987.
14. Metcalf, D. (1985) *Science* **229**, 16–22.
15. Golde, D. W. & Takaku, F. (1985) *Hematopoietic Stem Cells* (Dekker, New York).
16. Ihle, J. N., Keller, J., Oroszean, S., Henderson, L. E., Copeland, T. D., Fitch, F., Prystowsky, M. B., Goldwasser, E., Schrader, J. W., Palaszynski, E., Dy, M. & Lebel, B. (1983) *J. Immunol.* **131**, 282–287.
17. Burgess, A. W., Camakaris, J. & Metcalf, D. (1977) *J. Biol. Chem.* **252**, 1998–2003.
18. Stanley, E. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2969–2973.
19. Nicola, N. A., Metcalf, D., Matsumoto, M. & Johnson, G. R. (1983) *J. Biol. Chem.* **258**, 5290–5299.
20. Wong, G. G., Witek, J. S., Temple, P. A., Wilkens, K. M., Leary, A. C., Luxenberg, D. P., Jones, S. S., Brown, E. L., Kay, R. M., Orr, E. C., Shoemaker, C., Golde, D. W., Kaufman, R. J., Hewick, R. M., Wang, E. A. & Clark, S. C. (1985) *Science* **228**, 810–815.
21. Kawasaki, E. S., Ladner, M. B., Wang, A. M., Van Arsdell, J., Warren, M. K., Coyne, M. Y., Schweickart, V. L., Lee, M.-T., Wilson, K. J., Boosman, A., Stanley, E. R., Ralph, P. & Mark, D. F. (1985) *Science* **230**, 291–296.
22. Nagata, S., Tsuchiya, M., Asano, S., Kaziro, Y., Yamazaki, T., Yamamoto, O., Hirata, Y., Kuboto, N., Oheda, M., Nomura, H. & Ono, M. (1986) *Nature (London)* **319**, 415–418.
23. Souza, L. M., Boone, T. C., Gabrilove, J., Lai, P. H., Zsebo, K. M., Murdock, D. C., Chazin, V. R., Bruszewski, J., Lu, H., Chen, K. K., Barendt, J., Platzer, E., Moore, M. A. S., Mertelsmann, R. & Welte, K. (1986) *Science* **232**, 61–65.
24. Huebner, K. M., Isobe, M., Croce, C. M., Golde, D. W., Kaufman, S. E. & Gasson, J. C. (1985) *Science* **230**, 1282–1285.
25. Sherr, C. J., Rettenmier, C. W., Sacca, R., Roussel, M. F., Look, A. T. & Stanley, E. R. (1985) *Cell* **41**, 665–676.
26. Nienhuis, A. W., Bunn, H. F., Turner, P. H., Gopal, T. V., Nash, W. G., O'Brien, S. J. & Sherr, C. J. (1985) *Cell* **42**, 421–428.
27. Roussel, M. F., Sherr, C. J., Barker, P. E. & Ruddle, F. H. (1983) *J. Virol.* **48**, 770–773.
28. Le Beau, M. M., Westbrook, C. A., Diaz, M. O. & Rowley, J. D. (1984) *Nature (London)* **312**, 70–71.
29. O'Brien, S. J., Simonson, J. M. & Eichelberger, M. (1982) in *Techniques in Somatic Cell Genetics*, ed. Shay, J. W. (Plenum, New York), pp. 342–370.
30. Lemons, R. S., Nash, W. G., O'Brien, S. J., Benveniste, R. E. & Sherr, C. J. (1978) *Cell* **14**, 995–1005.
31. Feinberg, A. & Vogelstein, B. (1984) *Anal. Biochem.* **137**, 266–267.
32. Shima, E. A., Le Beau, M. M., McKeithan, T. W., Minowada, J., Showe, L. C., Mak, T. W., Minden, M. D., Rowley, J. D. & Diaz, M. O. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3439–3443.
33. Groffen, J., Stephenson, J. R., Heisterkamp, N., de Klein, A., Bartram, C. R. & Grosveld, G. (1984) *Cell* **36**, 93–99.
34. Murphree, A. L. & Benedict, W. F. (1984) *Science* **223**, 1028–1033.
35. Friend, S. H., Bernards, R., Rogelj, S., Weinberg, R. A., Rapaport, J. M., Albert, D. M. & Dryja, T. P. (1986) *Nature (London)* **323**, 643–646.
36. Lang, R. A., Metcalf, D., Gough, N. M., Dunn, A. R. & Gonda, T. J. (1985) *Cell* **43**, 531–542.
37. Yarden, Y., Escobedo, J. A., Kuang, W.-J., Yang-Feng, T. L., Daniel, T. O., Tremble, P. M., Chen, E. Y., Ando, M. E., Harkins, R. N., Francke, U., Fried, V. A., Ullrich, A. & Williams, L. T. (1986) *Nature (London)* **323**, 226–232.
38. Kobilka, B. K., Dixon, R. A. F., Frielle, T., Dohlman, H. G., Bolanowski, M. A., Sigal, I. S., Yang-Feng, T. L., Francke, U., Caron, M. G. & Lefkowitz, R. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 46–50.
39. Jaye, M., Howk, R., Burgess, W., Ricca, G. A., Chiu, I.-M., Ravera, M. W., O'Brien, S. J., Modi, W. S., Maciag, T. & Drohan, W. N. (1986) *Science* **233**, 541–545.
40. Eighth International Workshop on Human Gene Mapping (1985) *Cytogenet. Cell Genet.* **40** (1–4).