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TEL and *KIP1* Define the Smallest Region of Deletions on 12p13 in Hematopoietic Malignancies

By Yuko Sato, Yoshimasa Suto, Jennifer Pietenpol, Todd R. Golub, D. Gary Gilliland, Elizabeth M. Davis, Michelle M. Le Beau, James M. Roberts, Bert Vogelstein, Janet D. Rowley, and Stefan K. Bohlander

Unbalanced translocations as well as interstitial deletions of the short arm of chromosome 12 [del(12p)] are found as recurring chromosomal changes in a broad spectrum of hematopoietic malignancies. These changes result in the hemizygous deletion of genetic material from 12p. We mapped a yeast artificial chromosome containing the *TEL* gene, a cosmid contig containing part of *TEL* and a P1 contig containing the *KIP1* gene to 12p13. These probes were used for fluorescence in situ hybridization to analyze samples from 47 patients with various hematologic malignancies who had unbalanced translocations (25 patients) leading to loss of 12p or deletions (22 patients) involving 12p13. The patients had acute lymphoblastic leukemia (8 cases), myelodysplastic syndrome (MDS; 11 cases), acute myeloid leukemia (AML; 10 cases), myeloproliferative disorders (4 cases), therapy-related MDS or AML (7 cases), non-Hodgkin's lymphoma (2 cases), and other hematopoietic malignancies (5 cases). All three probes were hemizygously deleted in 26 cases and were completely retained in only 9 cases. In 12 cases probes for one of the two genes were deleted, allowing us to map the smallest region of overlap of these deletions to a small genomic region that is bordered on the telomeric side by the *TEL* gene and on the centromeric side by *KIP1*. The genomic distance between *TEL* and *KIP1* is estimated to be about 1 to 2 Mbp.

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BALANCED AND UNBALANCED translocations as well as interstitial deletions of the short arm of chromosome 12 are found as recurring abnormalities in both myeloid and lymphoid malignancies. Although several recurring 12p13 translocations have been identified [ie, t(5;12)(q33;p13),[1] t(7;12)(q11;p12-13),[2] t(12;13)(p13;q14),[3] t(12;17)(p13;q21),[4] t(12;21)(p12;q22),[5] and t(12;22)(p13;q12),[6]] a number of other chromosomal bands (6q15, 7q11, 7q36, 12q13, 12q22, and 22q11) have also been reported to be rearranged with 12p13 in various hematologic disorders.[7] We showed that in the majority of balanced translocations, the breakpoint on 12p13 occurs within a yeast artificial chromosome (YAC 964c10) that also contains the *TEL* gene.[8] *TEL* forms a fusion transcript with the platelet-derived growth factor receptor-β gene on 5q33 in patients with chronic myelomonocytic leukemia and a t(5;12)(q33;p13).[1] *TEL* (translocation ets leukemia) has an *ETS* DNA-binding domain and as well as a predicted helix-loop-helix domain (HLH) and is a member of the *ETS* family of transcription factors. Recent studies by us have shown that *TEL* also forms fusion transcripts with a number of other genes, including *ABL*[9,10] and *AML1*.[11] It is not known at present whether *TEL* is involved in all the of breakpoints that occur within YAC 964c10. The mechanism underlying the malignant transformation in the case of the TEL fusion proteins (TEL-PDGFRB, TEL-ABL, and TEL-AML1) is not known.

Recurring loss of genetic material from specific chromosomal regions suggests the presence of a tumor-suppressor gene in these regions. Deletions affecting band 12p13 and unbalanced translocations with breakpoints in 12p13, which both result in the loss of genetic material from the short arm of chromosome 12, are far more common than reciprocal translocations. In a recent review it was reported that the del(12p) is one of the most common deletions in both acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), suggesting that the involved gene plays a critical role in growth regulation of all hematopoietic cells.[12] Del(12p) is found in about 5% of childhood ALL cases,[3] but also in patients with myelodysplastic syndrome (MDS) and AML, both de novo and therapy related, and in myeloproliferative disorders (MPD).[7] The true frequency of del(12p) is probably higher because deletions may not be detectable by standard cytogenetic techniques. Loss of heterozygosity (LOH) on 12p was detected in 25% of ALL samples in a recent report.[13] Kobayashi et al[8] have recently shown that the telomeric border of a series of cytogenetically identified del(12p) samples maps very close to the breakpoints of the reciprocal translocations. The centromeric limits of the deletions could not be very well defined in this fluorescence in situ hybridization (FISH) study. Most of the patients showed large deletions that included the most centromeric marker (D12S120) that mapped to 12p12.1. In this study, the smallest region of overlap (SRO) of the deletions spanned a relatively large genomic region flanked by D12S133 (12p13.1) distally and by D12S140 (12p12.1) proximally, which probably encompasses 5 to 10 Mbp (Fig 1).

In the present study, we were able to define the SRO of these deletions further by studying patients with del(12p) or unbalanced translocations involving 12p13. The telomeric border of the smallest commonly deleted region is defined by the YAC 964c10 that contains the *TEL* gene and the centromeric border of the deletions is defined by a P1 contig[14] that contains the *KIP1* gene.[15,16] The protein encoded by *KIP1*, $p27^{KIP1}$, is an inhibitor of the cyclin E-Cdk2 complex and is thought to play an important role in the arrest of

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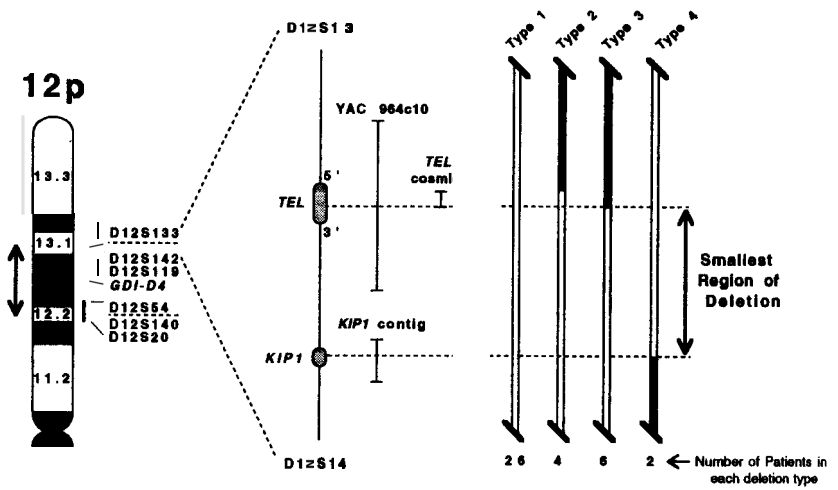


Fig 1. Diagrammatic representation of the extent of hemizygous deletions on the short arm of chromosome 12. The double-headed arrow on the left side of the chromosome ideogram shows the extent of the SRO, as defined by Kobayashi et al,[8] that is flanked by D12S133 and D12S140. The right-hand side of the diagram shows an expanded view of the region between D12S133 and D12S142 that contains *TEL* and *KIP1*, with the relative location of YAC 964c10 and the *TEL* cosmids and the *KIP1* contig. The open vertical bars symbolize hemizygously deleted regions in the different deletion types. The solid bars show homozygously retained regions. The smallest region of overlap of these deletions is defined by the type 3 and type 4 deletions. Even though in the type 3 neither the YAC probe nor *TEL* cosmids were deleted, the deletions could theoretically extend to include part of the *TEL* cosmids. Similarly, in the type 4 deletions, although a signal was seen with the *KIP1* probe, the telomeric portion of this probe could theoretically be deleted.

contact-inhibited and transforming growth factor-β-inhibited cells in the G1 to S phase transition.[17]

MATERIALS AND METHODS

Patients. Patients with hematologic malignant diseases and unbalanced translocations or deletions involving band 12p13 studied at the University of Chicago were selected for the present analysis. Patient samples were obtained with informed consent. Between 1970 and 1994, we identified 45 patients with unbalanced 12p13 translocations and 45 patients with deletions involving band 12p13. Cytogenetic material from 47 patients was available for FISH analysis (25 patients with unbalanced translocations and 22 patients with deletions). Eleven of these patients (nos. 11, 13, 14, 15, 18, 20, 24, 26, 30, 32, and 45) were previously reported by Kobayashi et al.[8] Clinical and cytogenetic data on these patients are summarized in Table 1.

Cytogenetic analysis. Metaphase cells were prepared from bone marrow or peripheral blood as previously described.[18] Thirty-two of the samples were obtained before treatment. The karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN 1991).[19]

FISH probes. Three FISH probes were used for the deletion analysis, ie, YAC 964c10, a cosmid contig containing part of the *TEL* gene, and a P1 contig containing *KIP1*. The CEPH YAC 964c10 has a human insert of 1,390 kb and contains the *TEL* gene as well as the sequence tagged sites (STSs) D12S89, D12S98, and D12S391.[20] We obtained the cosmids from a cosmid library constructed from YAC 964c10 that was screened with a 1.4-kb *TEL* cDNA clone containing the entire coding sequence of *TEL*.[1] The two cosmids in the contig cover approximately 50 kb and contain the middle portion of the *TEL* coding region. The P1 contig consisted of two phages (addresses: 2096 and 2097; Genome Systems, Inc), including the entire *KIP1* gene. In addition to these three probes, a cosmid, 1H9 (D12S142),[8,21] was used for the two-color FISH mapping of *KIP1*. The YAC and the 1H9 cosmid were kindly provided to us by Drs Kate Montgomery and Raju Kucherlapati (Albert Einstein College of Medicine, Bronx, NY). We also used centromere specific probes for chromosome 12 (CEP12 Spectrum Orange; Vysis, Framingham, MA), chromosome 13/21 and 14/22 (Chromosome 13/21 and Chromosome 14/22 Alpha Satellite DNA, digoxigenin-labeled; Oncor, Gaithersburg, MD), and whole chromosome painting probes for chromosome 7, 12, and X (Coatasome 7, Coatasome 12, and Coatasome X, digoxigenin-labeled; Oncor) to identify marker chromosomes or complicated rearrangements in some patients.

FISH. YAC964c10 was separated from the other yeast chromosomes with pulsed-field gel electrophoresis, the YAC band was excised, and the DNA was then sequence-independently amplified and labeled with biotin-11-dUTP, as previously described.[22] Ten nanograms of the P1 phage DNA were amplified and labeled similarly. The cosmids were labeled with biotin-11-dUTP or digoxigenin-11-dUTP using nick translation. The telomere to centromere order of YAC964c10 and the P1 contig was determined by two-color FISH analysis, as described previously.[23] The probes were hybridized to

Table 1. Clinical and Cytogenetic Data of the 47 Patients With Unbalanced Translocations or Deletions Involving 12p13 Band

Patient No.	Age/Sex	Diagnosis	Stage	Source	Karyotype
1	42/F	CLL	DX	BC	44,XX,−6,**dic(6;12)(p24;p13)**,der(17)t(16;17)(p13;p13)[6]/46,XX[7]
2	39/M	NHL-LB(T)	RL	BM	45,XY,t(1;2)(q32;p23),**der(12)t(12;18)(p13;q11.2)**,−18,add(19)(q13)[6]/ 44,idem,−18,der(19)t(18;?;19)(q11;?;q13)[11]/46,XY[3]
3	44/M	AML-M1	DX	BM	46,X,−Y,−2,add(4)(p11),add(5)(q15),del(5)(p11p15),+6,inv(11)(q13q25),**der(12)t(2;12)(q13;p13)**,−17,+2mar1[25]/47,idem,t(9;10)(q34;q22),+mar2[4]/46,XY[3]
4	49/M	AML-M1	DX	BM	46,XY,−7,+del(5)(q13q33),add(8)(q?),del(17)(p11),−12,**dic(12;17)(p13;p11)**,+mar1, +mar2[4]/44,idem,−13,−mar[8]/46,XY[1]
5	52/M	AML-M2	DX	BM	45,XY,−2,der(2)t(2;7;6)(p13;?;p21),del(3)(p13p25),−5,del(6)(p21p25),−7,i(8q),+i(8q), **der(12)inv(12)(p13q13)t(12;17)(q13;q11)**,−17,−17,+mar1+mar2, +mar3[4]/46,idem,+mar4[3]/46,idem,+3mar4[2]/related clones[9]
6	75/F	RAEB-T	DX	BM	46,XX,del(5)(q11q35),t(6;10)(p21.3;p15),−7,+8,**der(12)t(7;12)(p13;p13)**[1]/ 46,idem,t(16;20)(q13orq21;q13)[16]/unrelated clone[2]/46,XX[1]
7	66/M	RARS	RL	BC	45,XY,t(2;9)(p13;q22),del(5)(q12q33),−7,dic(14;21)(p13;p13),−15,add(20)(q11),add(22)(q11),add(22)(q11),+mar1,+mar2[12]/45,idem,add(3)(q21),der(4)t(3;4)(q21;q21),**der(12)t(4;12)(q27?;p13)**[9]
8	55/F	RAEB	DX	BM	46,XX,t(11;17)(q23;q25)[7]/46,idem,**add(12)(p13)**[7]/46,XX[2]
9	30/M	MDS	DX	PB	44,XY,del(3)(q12q29),del(5)(q22q33),−7,**add(12)(p13)**,−17,der(18)t(17;18)(q11;p11)[5]/ 42,idem,−3,−del(3)[6]/unrelated clones[7]
10	64/M	t-MDS	DX	PB	44,XY,add(1)(p36),der(?5)t(?5;?7)(q11;q11),−7,−17,add(20)(p11),add(21)(q22)[4]/ 44,idem,add(7)(q22)[8]/44,idem,del(4)(p11p15),−6,**add(12)(p13)**,del(13)(q14q21) or[8]
11	30/M	ALL	RL (BMT)	BM	46,XY,**del(12)(p11p13)**[4]/46,idem,del(6)(q13q22)[18]
12	13/M	ALL	DX	BM	46,XY,**del(12)(p12p13)**[6]/46,XY[15]
13	54/M	AML-M2	RL	BM	46,XY,**del(12)(p11p13)**[15]/46,XY[7]
14	57/F	AML-M4EO	RL	BM	48,XX,+8,**del(12)(p11p13)**,inv(16)(p13q22),+21[6]/46,XX[15]
15	85/F	AML-M6	DX	BM	46,XX,del(5)(q15q33orq21q34)[4]/46,idem,−7,+8,+11,**del(12)(p12p13)**,−15,del(16)(q13q24)[15]/46,XX[2]
16	60/F	t-MDS	RL	BM	45,XX,−7,der(5)t(5;7)(q12;p11)[1]/45,idem,**del(12)(p11p13)**[2]/ 45,idem,der(2)t(2;11)(q13;q14)ins(11;?)(q14;?),del(4)(q25q31)[17]
17	69/M	RA	RL	PB	45,XY,−7,**del(12)(p11p13)**[19]/44,idem,−13[2]
18	75/M	RAEB	DX	BM	46,XY,inv(15)(q13q24)[6]/46,idem,**del(12)(p11.2p13)**[14]/46,XY[4]
19	76/M	RAEB	RL	BM	46,XY,del(1)(p13p37?4),−5,t(5;14)(p15;q11),del(7)(q22q36),**der(12)del(12)(p12p13)del(12)(q22q24)**,del(18)(q21orq21q23),−20,+mar1,+mar2[7]/46,idem,del(8)(q13q22)[19]/46,XY[6]
20	92/F	MDS	DX	BM	47,XX,del(5)(q13q33),+8[6]/47,idem,**del(12)(p11p13)**[14]
21	74/M	MDS	DX	BM	46,XX,i(3)(q10),**add(5)(q13)**,**del(12)(p11p13)**[14]/92,idemx2[7]
22	56/F	t-DMS	RL	BM	44,XX,**del(12)(p11p13)**,add(12)(q13),dic(16;22)(q11;p11),−22[14]/ 46,XX,del(5)(q15q33),der(16)t(1;16)(q21;q27?4)[8]/46,XX[2]
23	60/F	MF	DX	PB PHA	46,XX,**del(12)(p11p13)**[12]/46,XX[3]
24	51/M	MF	DX	PB	46,XY,**del(12)(p11.2p13)**[20]
25	53/M	PV	DX	BM	46,XY,**del(12)(p12p13)**[11]/46,XY[9]
26*	81/M	AUL	DX	BM	46,add(X)(p11),Y,t(4;7)(q21;q22),**del(12)(p11p13)**[1]/45,idem,−3,−5,der(3)t(3;15)(q12;q15),t(9;12;14)(q34;p13;q22),der(15)del(15)(q11q15)t(3;15)(q12;q15),der(18)t(3;18)(q11;q12),+mar1[26]/45,XY,−3,−5,der(12)t(3;12)(q21;p13),der(19)t(3;19)(p21;q13),+mar2[3]/46,XY[1]
27	2/F	ALL	RL	BM	49,XX,**der(12)t(10;12)(q11;p13)**,+16,+18,+mar[14]/48,XX[6]
28	68/M	RAEB	DX	BM	47,XY,+8[3]/45,X,−Y,add(2)(q37?4),−5,−7,+8,add(8)(p21),add(11)(p1?3),der(12)t(12;17)(p12;q11),−17,**der(18)t(12;18)(p13;p11)**,+mar1,+mar2[10]/ 45,idem,add(10)(p13),−add(11),−mar2[6]
29	72/F	RAEB-T	DX	BM	45,XX,der(5)t(5;7)(q11.2;p11),−7,+8,der(12)del(12)(p12.1p13.1)t(12;14)(p13;q11)del(12)(q13q15),**der(14)t(12;14)(p13.1;q11)**,−16[13]/46,XX[8]
30	45/M	t-AML	DX	BM	44,XY,der(3;6)(q10;p10),del(5)(q11q34),−7,**del(12)(p11p13)**[4]/45,idem,+r[20]
31	15/M	ALL	RL	BM	46,XY,del(6)(q12?5q23?),t(8;11)(q24;p13),**der(12)t(12;22)(p13;q11)**,−22,+mar1[13]/ 47,XY,del(6),**t(5;12)(q21?5;p13)**,t(8;11),+mar1[6]/46,XY[3]
32*	21/F	ALL-L3	DX	BM	47,XX,**del(12)(p11p13)**,+**del(12)(p11p13)**,t(12;22)(p13;q13)[17]/46,XX[7]
33*	4/F	NHL	DX	PF	47,X,der(X)del(X)(q27?6;q27?6)t(X;12)(q27?6;p13),**del(12)(p11p13)**,der(12)t(X;12)(q27?6;p13),+21[13]/47,idem,add(13)(q32)[14]
34	53/F	AML-M6	DX	BC	44,XX,del(5)(q22q35),−7,add(9)(p27?),**del(12)(p11p13)**,−17,−18,der(19)t(7;19)(p12;p13),+mar1[7]/45,idem,+mar2[7]/46,XX[6]
35	68/F	t-AML	DX	BM	46,XX,del(5)(q13q33),del(9)(q12q32),**del(12)(p11p13)**[21]/46,XX[2]

Table 1. Clinical and Cytogenetic Data of the 47 Patients With Unbalanced Translocations or Deletions Involving 12p13 Band (Cont'd)

Patient No.	Age/Sex	Diagnosis	Stage	Source	Karyotype
36	47/M	MF	DX	PB	47-50,XY,-5,del(7)(q31q35),+8,+11,**der(12)t(12;15)(p13;q?2)**,add(13)(p11),r(?13),-15,-15,add(21)(q22),+2-5mars[8]/46,XY[7]
37	81/F	AL	DX	BM	43,XX,**der(2)del(2)(q21q31)t(2;12)(q37;p13)**,-5,-10,der(12)t(10;12)(q11;p11),-13,-17,-21,-22,+mar1,+mar2,+mar3[3]/43,idem,add(8)(p23),+16,add(16)(q24),-mar3[13]/46,XX[3]
38	47/F		DX	BM	44,XX,-5,r(7)(p22q11),dic(11;15)(p11;p11),**add(12)(p13)**,add(16)(q13),dup(19)(q11q13.1),-21,-22,+mar1,+mar2[21]/45,idem,+mar3[3]/45,idem,+r(7)[3]/46,XX[3]
39	7/M		RL (BMT)	BM	45,XY,der(5)t(5;8)(q13;q1?3),-8,t(9;17)(q32;q11),**add(12)(p13)**,add(18)(q21)[7]/5 unrelated clones[12]/46,XY[3]
40	50/F	ALL	⊠	BM	46,XX,**add(12)(p13)**[14]/46,XX[2]
41	3/M	ALL	⊠	BM	46,XY,+X,der(7)del(7)(p15p22)t(7;12)(q31;p13),der(8)t(8;9)(p21;p21)t(8;17)(q24;q11),der(9)t(8;9)(p21;p21),**der(12)t(7;12)(q31;p13)**,-17[21]/46,XY[2]
42	44/M	AML-M2	RL	☺	92,XXYY,+Y,del(1)(p3?3p3?5),+2,t(2;2)(q35;q37),der(6)t(6;15)(p23;q21.2orp21;q15),t(7;16)(p?2;p13),2t(8;21)(q22;q22),-11,**der(12)t(12;15)(p13;q?3)**,-15,-15,add(16)(p13),-17,del(17)(q2?1q2?3),+18,der(19)t(11;19)(q13;q13),2t(21;22)(q11;q13),+mar1[4]/94,idem,+mar2,+mar3[3]/46,XY[12]
43	60/M	AML-M2	RL	☺	46,XY,del(7)(q21q36),**der(12)t(3;12)(p21;p13)**[4]/46,XY,r(7)(p22q34),-12,+mar[5]/46,XY[13]
44	48/M	t-AML	⊠	☺	45,XY,del(6)(q11q36),add(7)(q11),add(11)(p11),**add(12)(p13)**,-21[13]/5 related clones[12]
45	79/M	t-AML	BI	☺	45,XY,der(2)del(2)(q2?3q3?5)add(2)(p13)t(2;12)(q37;p13),del(3)(p14p23),-4,del(5)(q13q34orq35),-6,-7,**der(12)del(12)(p11p13)t(2;12)(q37;p13)**,+der(?)t(?;6)(?;p21),+mar1[2]/2 related clones[5]/46,XY[11]
46	36/M	CML	AP	BM	46,XY,t(9;22)(q34;q11),**der(12)t(2;12)(p14;p13)**,add(18)(p11)[23]
47	46/F	AML-M1	DX	BM	47,XX,+10,**del(12)(p12p13)**[32]/46,XX[1]

Patients no. 11, 13, 14, 15, 18, 20, 24, 26, 30, 32, and 45 are patients no. 14, 15, 19, 20, 17, 18, 16, 4, 21, 11, and 12 in Kobayashi et al,[8] respectively.

Abbreviations: AL, acute leukemia; AUL, acute undifferentiated leukemia; MF, myelofibrosis; NHL-LB(T), non-Hodgkin's lymphoma-lymphoblastic (T-cell origin); RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess blasts; RAEB-T, RAEB in transformation; PV, polycythemia vera; DX, diagnosis; RL, relapse; AP, acute phase; BM, bone marrow sample; BC, bone core sample; PB, peripheral blood; PB-PHA, PB phytohemagglutinin stimulated.

* Patients with a del(12) and a der(12) and no normal chromosome 12. The deletions and unbalanced translocations involving 12p13 are in boldface; reciprocal translocations involving 12p13 are underlined.

patient slides, as previously described.[24] Chromosomes were identified using counterstaining with 4′ 6-diamidino-2-phenylindole dihydrochloride (DAPI). The presence or absence of the FISH signals was scored on an average of 12 abnormal metaphase cells (range, 2 to 20 cells) per probe per patient by two persons blinded to the identity of probes and patients.

Images of the hybridizations were captured with a liquid-cooled, charge-coupled device camera (Photometrics, Tucson, AZ). Separate gray-scale images for the DAPI and the fluorescein isothiocyanate (FITC) fluorescence were acquired. After adjusting the gray levels with the NIH image 1.52 software (National Institutes of Health, Bethesda, MD), the images were merged in RGB format using Adobe Photoshop on a Macintosh computer (MacIntosh-Apple Corp, Cupertino, CA).

RESULTS

Ordering of probes. The P1 contig containing *KIP1* was mapped to the region between *TEL* and D12S142 by two-color FISH on normal metaphase chromosomes (tel-*TEL*-*KIP1*-D12S142-cen; Fig 1). D12S142 was described previously as the closest marker centromeric to YAC 964c10 by Kobayashi et al.[8]

FISH analysis of patient material. The FISH results on the patients with unbalanced 12p13 translocations or deletions are summarized in Table 2. FISH analysis was performed with YAC 964c10 and the *KIP1* contig. In those cases in which the YAC was retained, further analysis was performed using the *TEL* cosmids.

In patients no. 1 through 26, YAC964c10 and the *KIP1* P1 contig were hemizygously deleted (11 unbalanced translocations and 15 deletions). Signals for these probes were found only on the normal chromosome 12 but not on the der(12) or del(12) chromosome. We called this a type 1 deletion (Figs 1 and 2A and B).

In 4 patients (no. 27 through 30), the *TEL* cosmids and the *KIP1* contig were hemizygously deleted, whereas two signals were detected for YAC 964c10 (type 2 deletion; Figs 1 and 2C through E). In 3 cases (patients no. 27 through 29) with unbalanced translocations, the YAC signal was found not on the der(12) chromosome but rather on other chromosomes, ie, on a marker chromosome, on chromosome 2, and on a der(14), respectively. Thus, these patients actually have translocations accompanied by deletions of DNA; the telomeric breakpoint of the deletion/translocation must be located within YAC 964c10.

In the 6 type 3 deletion cases (patients no. 31 through 36), only the *KIP1* contig signal was hemizygously deleted, whereas both the YAC 964c10 and the *TEL* cosmid signals

Table 2. Results of FISH Analysis Using the YAC 964c10, the *TEL* Cosmid, and the *KIP* Probes in 47 Patients With Unbalanced Translocations or Deletions Involving Band 12p13

		Location of FISH Signals					
		YAC 964c10 Probe		*TEL* Cosmid Probe		*KIP1* Probe	
Patient No.	Deletion Type	Other Chromosomes	ni.12	Other Chromosomes	ni.12	Other Chromosomes	ni.12
1-25	1 (N = 28)	—	+	ND	ND	—	+
26*		—	+†	—	+†	—	+†
27	2 (N = 4)	+mar	+	—	+	—	+
28		+2qter	+	—	+	—	+
29		+der(14)	+	—	+	—	+
30		+del(12)	+	—	+	—	+
31	3 (N = 6)	+der(21), +mar	+	+der(21), +mar	+	—	+
32*		+del(12), +del(12)	+†	+del(12), +del(12)	+†	—	+†
33*		+del(12)	+†	+del(12)	+†	—	+†
34		+del(12), +add(9), +mar	+	+mar	+	—	+
35		+del(12)	+	+del(12)	+	—	+
36		+der(12)	+	+der(12)	+	—	+
37	4 (N = 2)	—	+	ND	ND	+der (12), +der(2)cen	+
38		—	+	ND	ND	+add(12)	+
39	No deletion (N = 9)	+F-group	+	ND	ND	+add(12)	+
40		+add(12)	+	+add(12)	+	+add(12)	+
41		+der(12)	+	+der(12)	+	+der(12)	+
42		+der(12)	+	+der(12)	+	+der(12)	+
43		+der(12)	+	+der(12)	+	+der(12)	+
44		+add(12)	+	+add(12)	+	+add(12)	+
45		+der(2)	+	+der(2)	+	+der(2)	+
46		+der(12)	+	+der(12)	+	+der(12)	+
47		+del(12)	+	+del(12)	+	+del(12)	+

Abbreviations: ND, not done; +, a signal is present on specified chromosome; -, no signal was present on any of the other chromosomes.

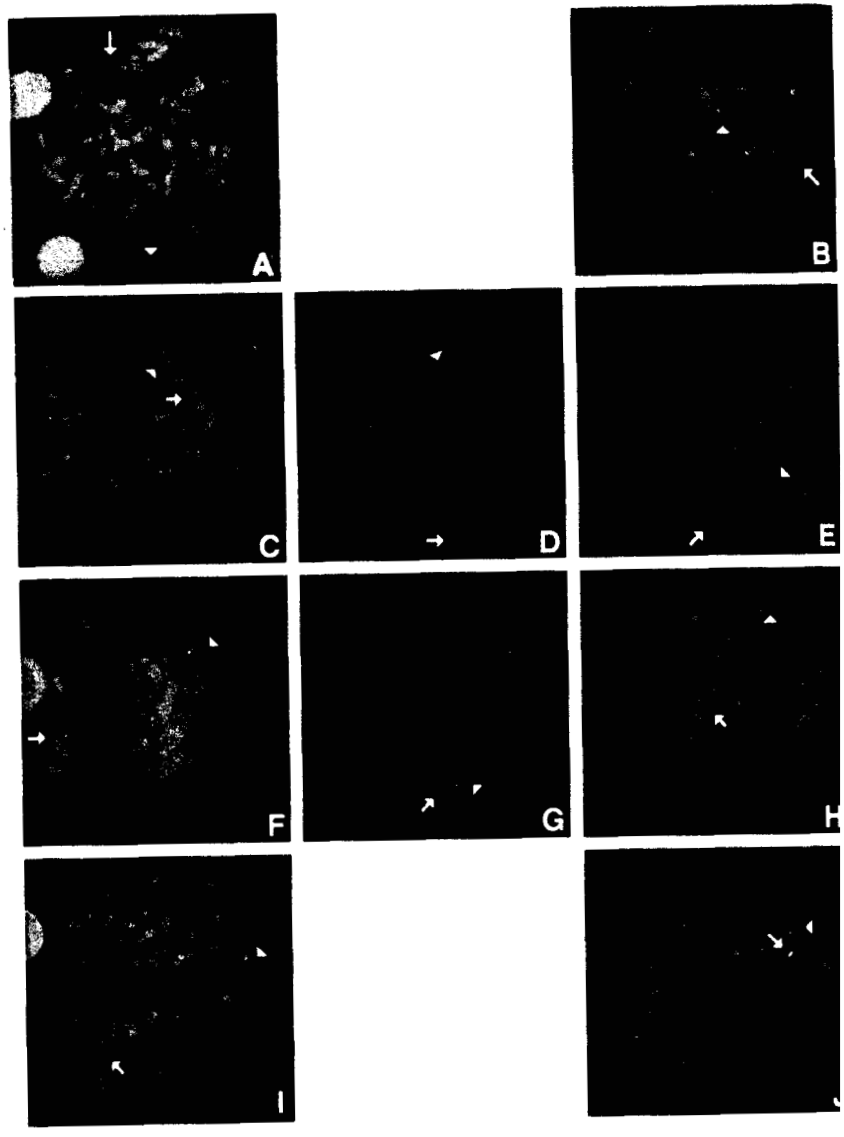
* Patients with a del(12) and a der(12) and no normal chromosome 12.

† The three probes were retained on the der(12) in patients no. 26 and 32 and on Xqter in patient no. 33.

were retained (Figs 1 and 2F through H). In 2 of these 6 cases (patients no. 31 and 34), additional FISH signals were detected with the YAC and *TEL* cosmid probe. Patient no. 31 had two clones, ie, a major clone with a der(12) t(12;22)(p13;q11) and a minor clone with a t(5;12)(q?15;p13). In the major clone, three YAC and *TEL* cosmid contig signals were detected in each metaphase: one on the normal chromosome 12, one on a marker chromosome, and one on a cytogenetically normal chromosome 21. In the minor clone with the t(5;12)(q?15;p13), three YAC and *TEL* cosmid contig signals were detected: one on the normal chromosome 12, one on the der(5), and one on the der(12). In patient no. 34, four YAC signals and two *TEL* cosmid contig signals were seen. The YAC signals were located on the normal 12, the der(12), an add(9), and a marker chromosome. The two *TEL* cosmid contig signals were detected only on the normal 12 and the marker chromosome. In patients no. 31 and 34, the *KIP1* P1 signal was only present on the normal chromosome 12. These findings imply very complicated rearrangements in which multiple chromosomal breaks must have occurred in a small genomic region on 12p as well as on other chromosomes. The *TEL* gene is oriented 5′ to 3′, telomere to centromere, on 12p.[1] We cannot exclude that these type 3 deletions affect the 3′ portion of *TEL*, because our cosmid contig does not contain the 3′ portion of *TEL* (Fig 1). Moreover, a small deletion of the *TEL* cosmid contig would not be detected in our FISH analyses.

In the 2 cases with type 4 deletions (patients no. 37 and 38), the YAC signal was hemizygously deleted, whereas the *KIP1* P1 contig signal was retained on both chromosome 12 homologues (Figs 1 and 2I and J). We cannot exclude the possibility that these deletions affect *KIP1* because a FISH signal would still be visible if half or more of the DNA corresponding to the P1 contig was deleted. In patient no. 37, three *KIP1* P1 signals were observed: one on the normal chromosome 12, one on the der(12), and one near the centromere of a der(2).

In 9 patients (no. 39 through 47) with rearrangements affecting 12p13 (8 unbalanced translocations and 1 deletion), none of the probes used in this study was deleted. Patient no. 39 probably has a balanced translocation, because the YAC 964c10 signal was found on a F-group chromosome, whereas the *KIP1* signal remained on the add(12). In the remaining 8 patients, the three FISH probes were located on the same derivative chromosomes. These findings suggest that, in the patients with the unbalanced translocations (patients no. 40 through 44 and 46), the deletions affect the more distal portion of 12p13, whereas in patient no. 45, the deleted region is centromeric to *KIP1* as was shown by Kobayashi et al.[8]



Based on these data, the SRO for these ... between *TEL* and *KIP1*. As noted earlier, we cannot exclude the possibility that the deletions include the 3′ end of the *TEL* gene and/or part of the *KIP1* gene as shown in Fig 1. However, it should be noted that we have observed deletions of 12p13 that apparently do not overlap this SRO.

No obvious correlation between the type of malignancy and the extent of the deletions was noted. Thirty-three patients had disorders affecting the myeloid lineage, including MDS (11 cases; t-MDS, 3 cases), AML (10 cases), therapy-related AML (t-AML; 4 cases), chronic myeloid leukemia (CML; 1 case), or MPD (4 cases). Eleven patients had lymphoid disorders, including ALL (8 cases), chronic lymphocytic leukemia (CLL; 1 case), and non-Hodgkin's lymphoma (2 cases). Two patients had acute undifferentiated leukemia and 1 patient had an unspecified acute leukemia. Ten patients had simple karyotypes with one or two numerical or structural abnormalities (nos. 8, 11, 12, 13, 17, 22, 23, 24, 32, 40, and 47). Most cases had complex abnormal karyotypes with more than three numerical or structural abnormalities. In addition, these patients often had multiple clones that had related karyotypic changes. In the majority of these patients, the abnormal 12p was found in the simplest clone and was thus likely to be an early event. In 6 other patients, the 12p abnormality was a secondary event. Finally, 4 patients (no. 22, 31, 39, and 43), all of whom were studied only in relapse, had two or more independent clones with no common abnormalities. In 1 patient (no. 31), both clones involved 12p13, and in 1 other patient (no. 43), the second clone was −12, +mar; therefore, involvement of 12p13 in both clones cannot be excluded. Of 5 patients whose sole karyotypic abnormality was a del(12p) including the *TEL-KIP1* region, 1 had ALL, 1 had AML, and 3 had a myeloproliferative syndrome (2 with myelofibrosis and 1 with polycythemia vera).

DISCUSSION

In the present study we were able to define more precisely the SRO of hemizygous deletions on 12p13. By using patient samples that had unbalanced translocations as well as deletions it was possible to restrict the SRO to a small genomic segment bordered by *TEL* on the telomeric side and by *KIP1* on the centromeric side. The genomic distance between these two genes cannot be more than 2.9 Mbp because *KIP1* is contained within YAC 954g10 that overlaps YAC 964c10 that contains *TEL* (S.K.B., unpublished observation; K.T. Montgomery, personal communication, October 1994), and their combined size is about 2.9 Mbp. The actual distance between *TEL* and *KIP1* is ... unfortunately, the FISH probes we used did not allow us to exclude either one of these two genes from the deleted region. In the type 3 deletions (Fig 1) in which we found loss of the *KIP1* P1 phage only, part of YAC964c10 and/or the *TEL* cosmid contig and thus the 3′ end of *TEL* could have been deleted. The *TEL* cosmid contig spans only about 50 kb of genomic sequence that corresponds to the middle portion of the coding sequence of *TEL*. Similarly, in the type 4 deletions in which only the *KIP1* P1 phage is retained and both the YAC and the *TEL* cosmid contig are deleted, part of the *KIP1* P1 phage could be deleted, possibly including *KIP1* itself. Clearly, further studies with probes that map between *TEL* and *KIP1* are needed to determine the true extent of the hemizygous deletions in the 8 cases with type 3 and type 4 deletions. Interestingly, we have not found any evidence for homozygous deletions of 12p13 by FISH. This is quite different from the situation involving band 9p21 in which the two putative tumor-suppressor genes *CDKN2* and the gene for $p15^{INK4B}$ together with large genomic regions are frequently homozygously deleted.[25,26] It should also be noted that we observed deletions of 12p13 that affect regions both distal and proximal to the *TEL-KIP1* interval.

Although the involvement of *TEL* in fusion genes indicates that it may act in a dominant fashion, both *TEL* and *KIP1* are candidate tumor-suppressor genes because they may map to the SRO that we have defined on 12p. Tumor suppressor genes function as negative regulators of cell growth. According to Knudson's classical two-hit model both alleles of a tumor-suppressor gene must be inactivated to initiate a tumor or to allow a tumor to progress.[29]

The *KIP1* gene product, $p27^{KIP1}$, is an inhibitor of the cyclin E-Cdk2 complex.[15-17] $p27^{KIP1}$ is involved in mediating the signals from contact inhibition and transforming growth factor-β that result in cell cycle arrest in late G1 phase.[17] It has also been shown that interleukin-2 treatment inactivates $p27^{KIP1}$ in T lymphocytes, which can then progress from G1 to S phase.[30] These findings suggest that $p27^{KIP1}$ is likely to play an important negative role in the growth-regulatory pathways of at least some hematopoietic cell lineages. These observations make *KIP1* an excellent candidate tumor-suppressor gene.

One copy of *KIP1* was deleted in 75% of our tumor samples. To examine the possibility that the remaining allele is inactivated by a point mutation, the coding region and exon-intron boundaries of the remaining allele were sequenced in 8 of our patients (no. 3, 15, 16, 18, 20, 26, 30, and 32). No inactivating mutations of *KIP1* were found in these

Fig 2. Metaphase cells from 4 patients representing the four types of deletions hybridized with the YAC 964c10 probe (left column), the *TEL* cosmid contig probe (middle column), and the *KIP1* P1 contig probe (right column). The arrowhead points to the normal chromosome 12 homologue and the arrow to the rearranged chromosome 12 [del(12) or der(12)]. (A and B) Type 1 deletion. Metaphase cells from patient no. 10 showing hemizygous deletions for both YAC 964c10 (A) and the *KIP1* probe (B). (C through E) Type 2 deletion. Metaphase cells from patient no. 30 showing two YAC 964c10 signals (C) but hemizygous deletions of the *TEL* cosmid probe (D) and the *KIP1* probe (E). (F through H) Type 3 deletion. Metaphase cells from patient no. 36 showing retention of two YAC (F) and two *TEL* cosmid contig signals (G) but hemizygous deletion of the *KIP1* probe (H). The second YAC and *TEL* cosmid signal is found near the centromere of a der(2) chromosome (arrow in F and G). (I and J) Type 4 deletion. Metaphase cells from patient no. 38 showing hemizygous deletion of the YAC 964c10 probe (I) and homozygous retention of the *KIP1* probe (J).

samples. Although this is not what one would have expected for a classical tumor-suppressor gene, the hemizygous deletions reduce the gene dosage of *KIP1* by 50%, which could relax the control of the G1- to S-phase transition.

TEL encodes a predicted 452 amino acid protein with a 5′ HLH domain and a 3′ *ETS* DNA-binding domain.[1] The 3′ *ETS* DNA-binding domain is conserved among all *ETS* family members.[31] However, the 5′ HLH domain is found in only 8 other family members (*ETS1, ETS2, ERG, FLI1, ELG, E4TF1-60, GABP-α*, and the *Drosophila* gene *yan/pok*).[31-33] *TEL* probably acts either alone or in concert with other proteins as a transcription factor. The target genes that are regulated by *TEL* and whether *TEL* suppresses or activates those genes are unknown. Interestingly, the amino acid sequence of the 5′ HLH domain and the 3′ DNA-binding domain of *TEL* is more closely related to the product of the *Drosophila* gene *yan/pok* than to the corresponding domains of any human *ETS* family member.[34,35] Whereas most *ETS* family members that have been studied in detail act as activators of transcription,[31] it has recently been shown in cultured insect cells that the *yan/pok* gene product represses the transcriptional activity of *pointed*, another member of the *ETS* family.[36] Thus, the *TEL* gene could normally function as a negative regulator of transcription, which would make *TEL* a candidate tumor-suppressor gene. Whether the remaining *TEL* allele in the hemizygous deletions has mutations is unknown. However, in several leukemic patient samples with reciprocal translocations involving *TEL*, the remaining *TEL* allele is deleted. Thus, no normal TEL protein could have been present in the leukemia cells, because one allele was deleted and the other allele gave rise to a fusion protein (eg, TEL-ABL[10] and TEL-AML1[11]). In these cases it is difficult to distinguish between the effects resulting from the complete absence of a normal *TEL* gene product and the effects of the fusion protein. To confirm the role of *TEL* as a tumor-suppressor gene in our series of hemizygous deletions, we will examine the remaining *TEL* allele for mutations.

A further explanation for the deletion patterns observed is that there is more than one target. One target could be *TEL* or a gene telomeric to *TEL;* the other could be *KIP1* or a gene centromeric to *KIP1*. Finally, there is the possibility that an as yet unidentified gene lies in the interval between *TEL* and *KIP1* and that it is the important tumor-suppressor gene in that region. Further studies with FISH probes that lie between *TEL* and *KIP1* are underway to resolve this uncertai

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