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Deletions of *PURA*, at 5q31, and *PURB*, at 7p13, in myelodysplastic syndrome and progression to acute myelogenous leukemia

K Lezon-Geyda[1,2], V Najfeld[2] and EM Johnson[1]

[1]*Departments of Pathology, Biochemistry and Molecular Biology, the Derald H Ruttenberg Cancer Center; and* [2]*Tumor Cytogenetics Laboratory, Polly Annenberg Levee Hematology Center, Mount Sinai School of Medicine, New York, NY, USA*

Deletions or monosomy of chromosomes 5 and 7 are frequently observed in myelodysplastic syndromes (MDS) and acute myelogenous leukemia (AML). In this study two genes, *PURA* and *PURB*, encoding functionally cooperative proteins in the Pur family, are localized to chromosome bands 5q31.1 and 7p13, respectively. One or both of these loci are shown to be hemizygously deleted in 60 MDS or AML patients using fluorescence *in situ* hybridization (FISH). High-resolution mapping of *PURA* localizes it approximately 1.1 Mb telomeric to the *EGR-1* gene. Frequency of *PURA* deletion and segregation with *EGR-1* indicate that *PURA* is within the most commonly deleted segment in myeloid disorders characterized by del(5)(q31). No mutations have been detected within the coding sequence of *PURA*. Concurrent deletions of *PURA* and *PURB* occur in MDS at a rate nearly 1.5-fold higher than statistically expected and in AML at a rate 5-fold higher. This novel simultaneous deletion of two closely related gene family members may thus have consequences related to progression to AML. Pur , an Rb-binding protein, has been implicated in cell cycle control and differentiation, and Pur and Pur are reported to function as heterodimers. Alterations in these genes could affect a delicate balance critical in myeloid development. *Leukemia* (2001) **15,** 954–962.
Keywords: Pur; MDS; AML; chromosome 5; chromosome 7; haploinsufficiency

Introduction

Monosomy of chromosomes 5 and 7 or deletion of their long arms [–5/del(5q); –7/del(7q)] are frequently observed chromosomal abnormalities in myeloproliferative disorders, and specifically in *de novo* MDS, in which each is observed in about 30% of the cases.[1–3] Therapy-related MDS (t-MDS) and therapy-related AML (t-AML) are associated with a higher frequency of karyotypic abnormalities, often including –5/del(5q) and –7/del(7q). Nearly 71% of patients with t-MDS/t-AML have abnormalities of chromosomes 5 and/or 7, and approximately 22% have abnormalities of both 5 and 7 simultaneously.[4,5]

In MDS and AML the deletions of the long arms of chromosome 5 are frequently interstitial and the breakpoints are variable.[6,7] The most commonly deleted segment of 5q in MDS, within band region 5q31, is a subject of considerable investigation.[5,8–11] Many genes involved in myeloid cell growth and differentiation have been mapped to band region 5q31, including *IRF1*,[12] *IL-9*, *EGR-1*, *CDC25C*,[5] *CD14*,[13] and *Smad5*.[14] It has frequently been speculated that the deletion of this chromosomal region leads to inactivation of one or more tumor suppressor genes. To date, however, none of the genes analyzed has been demonstrated to be a tumor suppressor of myeloid cell lineage. It thus remains possible that either deletion of genetic material leads to the imbalance of several important gene products,[15] or loss of function of a single gene product, the levels of which are critical, may be conferred by haploinsufficiency.[16] Similarly, no tumor suppressor genes have yet been identified at deleted loci on chromosome 7. The possibility has been noted that tumor suppressor genes are present at more than one site on the long arms of chromosome 7,[17] as deletions of 7q are more frequently seen in MDS patients than deletions in 7p.[1] On the other hand, deletions of 7p are seen in a significant number of cases,[1,18] and thus it is conceivable that the high prevalence of monosomy 7 reflects the critical nature of multiple genes on both the short and long arms of chromosome 7.

Pur is a family of sequence-specific single-stranded DNA-binding proteins that have an affinity for a purine-rich element.[19,20] Human Pur is encoded by the *PURA* gene, localized to chromosome 5, band region 5q31.[21] Expression of the *PURA* gene has been observed in all tissues analyzed, and the protein is highly conserved across several species.[22–24] Pur has been implicated in control of DNA replication,[22,25,26] and it has been reported to be a transcriptional activator of both viral[27] and mammalian[23,28,29] promoters.

A second family member, Pur , is more than 70% homologous to Pur ,[19] and Pur reportedly functions in a complex with Pur .[20] Binding of the Pur homodimer to the myelin basic protein gene promoter has been reported to activate transcription.[27] In contrast, binding of the Pur /Pur heterodimeric complex to the mouse vascular smooth muscle - actin gene enhancer results in transcriptional repression.[30] The kinetics of dimerization of these two proteins is very likely sensitive to any change in the intracellular levels of each. We report here that the human gene that encodes the Pur protein is mapped to the short arms of chromosome 7, band region 7p13.

For the present study we have analyzed samples from 60 patients with myeloid disorders. A high percentage of these patients have hemizygous deletions of either the *PURA* or *PURB* loci, and a substantial percentage have deletions of both loci. In the majority of patients with MDS the overall occurrence of simultaneous deletion of *PURA* and *PURB* loci are higher than predicted on the basis of occurrence of hemizygous deletion of either one individually. These data combined with evidence that the intracellular level of the Pur protein is critical for proliferation and differentiation render possible a haploinsufficiency phenomenon in which a complex of two cooperating proteins is affected.

Materials and methods

Patients, samples, and conventional cytogenetic findings

Fixed cytogenetic bone marrow samples from 90 individuals were selected and obtained following routine cytogenetic analysis in the Tumor Cytogenetics Laboratory at The Mount

Correspondence: K Lezon-Geyda, Box 1079, Tumor Cytogenetics, Mount Sinai School of Medicine, New York, NY 10029, USA; Fax: (212) 996–1029

Sinai Medical Center. Karyotypes were designated according to the International System for Human Cytogenetic Nomenclature.[31] Three groups of 30 individuals were identified for this study: (1) patients diagnosed with MDS or AML and identified to have normal karyotypes; (2) patients diagnosed with MDS or AML and identified to have abnormal karyotypes including abnormalities of chromosomes 5 and/or 7; and (3) healthy bone marrow transplantation donors. In order to investigate possible microdeletions of loci in band region 5q31, 30 of the 60 patients selected for this study possessed a normal karyotype. Of the 30, 22 had MDS and eight had AML. Of the 30 with abnormal karyotypes (Tables 1 and 2), 24 had MDS and six had AML. Nine patients (No. 17 and Nos 22–29) had concomitant abnormalities of chromosomes 5 and 7 as part of their complex karyotypes. Only three of the 60 patients had secondary disease due to therapy for other malignancies (Nos 24–26). The primary malignancy diagnosed for patient No. 24 was lymphoma and for patients Nos 25 and 26 it was breast cancer. Thirty additional samples from healthy bone marrow donors with normal cytogenetics were employed as controls for this study.

Bacterial artificial chromosome sources and preparation

Bacterial artificial chromosome (BAC) clones isolated from libraries constructed at Caltech[32,33] were obtained from three sources for this study. (Note that 'BAC' denotes a DNA clone and 'bac' denotes a polymorphic genomic marker.) Two BAC clones, 153O15 and 507K3, were obtained from Research Genetics (Huntsville, AL, USA) based on a screen of CITB Human BAC Resources for *PURA* by polymerase chain reaction (PCR). The PCR screen was done using primers specific for a 188 bp sequence of the human *PURA* gene. In addition two STS-BAC pairs (clones identified to contain a specific STS marker) D5S658 and D5S487, mapped to 5q, were obtained from the laboratory of Dr J Korenberg (Cedars- Sinai Medical Center, UCLA, Los Angeles, CA, USA). BAC clone GS025I23, which contains the 7p genomic marker sWSS1147,[34] also called STS D7S478, and which we reveal to contain *PURB*, was obtained from Genome Systems (St Louis, MO, USA). The BAC DNA was prepared as described by Hubert *et al*,[35] purified on Qiagen columns (Qiagen, Valencia, CA, USA), treated with RNase A (Roche Molecular Biochemicals, Indianapolis, IN, USA), and extracted with phenol/chloroform prior to labeling.

DNA probes for fluorescence in situ hybridization

Labeling of BAC DNA probes with biotin or digoxigenin (DIG) was performed using either Biotin or DIG Nick Translation Mix (Roche Molecular Biochemicals). The *EGR-1* LSI 5q31 probe in Spectrum Orange and Whole Chromosome Paint 5 (WCP 5) probe in Spectrum Green were obtained from Vysis (Downers Grove, IL, USA).

Table 1 Clinical and cytogenetic data of cases analyzed for loss of *PURA*

Patient	*S/A*	*Diagnosis*	*Karyotype*
1	F/75	AML	46,XX,**del(5)(q14q32)** [30]
2	F/66	MDS	46,XX,**del(5)(q12q31)** [13]/46,XX [8]
3	M/89	MDS	46,XY,**del(5)(q13q32)** [2]/46,idem,del(13)(q12q21) [9]/46,XY [12]
4	M/71	MDS	46,XY,**del(5)(q14q32)**,del(20)(q12) [22]/46,XY [6]
5	M/69	AML	42,XY,dic(1;2)(q23;q34),t(3;12)(p12;p13),**del(5)(q12q34)**,del(13)(q13q31),del(14)(q12q24)add(17)(p11),–17,–18,–20,add(22)(q13) [23]
6	M/49	MDS	43,XY,**del(5)(q12)**,t(7;11)(q11;p14),–12,–16,–20 [22]/46,XY [1]
7	M/71	MDS	46,XY,**del(5)(q13q31)**,inv(9)(p12q21) [12]/46,idem,add(19)(q13) [3]/46,idem,add(21)(p11) [1]/46,XY [7]
8	F/62	MDS	47,XX,t(1;17)(p36;q11),**del(5)(q12q31),**del(9)(q11q22)x2,+16,–21, +mar1 [1]/44,idem,–12,–13,–19 [1]/46,XX [2]
9	M/47	AML	45,XY,**del(5)(q13q35)**,add(6)(q27),add(6)(q27),add(7)(q36),–9,add(9)(p26),der(11),–13,+19,add(20)(q13),add(21)(q22),add(21)(q22) [29]
10	M/62	MDS	46,XY,t(1;16)(p13;p13),inv(3)(q21q26),**del(5)(q14q35)** [14]
11	M/74	MDS	51,XY,+1,**del(5)(q21q33)**,+9,+11,+14,+22 [30]
12	M/76	MDS	44,XY,t(1;6;15)(p31;q14;q14),–3,der(4)t(3;4)(q13;p16),–**5**,t(7;8)(q13;p23),del(11([14),–12,+mar [30]/46,XY [2]
13	M/5	MDS	46,XY,–**5**,t(10;11)(p14;q23),+mar [2]/46,XY [32]
14	F/67	MDS	49,XX,+X,–**5**,+6,del(7)(q22),+8,+10,+13,der(17)**t(5;17)(q11;p11)** [26]/46,XX [4]
15	F/45	MDS	46,XX,add(2)(q37),**del(5)(q31q34),del(5)(q14q34)**,del(7)(q22),del(9)(q22),–18,der(22),+mar [10]/46,XX [1]
16	F/72	AML	44,XX,der(2)t(2;11)(q33;q13),**del(5)(q31q35),del(5)(q14q35)**,+8,der(10)t(10;17)(p15;q21),–11,–17,–18 [30]/46,XX [1]
17	F/76	MDS	62,XXX,–3,–4,**del(5)(q14q35)x2**,der(6)t(1;6)(q21;q14),–**7**,r(11),–12,–15,–17,–18,der(20)t(1;20)(q21;q12),+21 [7]/46,XX [1]
18	F/68	MDS	46,XX,**t(1;5)(p21;q21)**,del(11)(q14) [7]
19	F/55	AML	46,XX,**t(3;5)(p13;q13)** [25]/46,idem,del(7)(q22) [3]/46,XX [2]
20	M/36	AML	44,XY,**der(5)t(5;14)(q11;q11)**,der(6)t(6;14)(p25;q11),–14,–14 [7]/46,XY [23]
21	M/66	MDS	44,XY,del(3)(q24q27),**der(5)t(5;?)(q11;?)**,–21 [2]/45,idem,–20 [18]

S, sex; A, age; M, male; F, female; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia.
Bold indicates an abnormality of chromosome 5.

Table 2 Clinical and cytogenetic data of cases analyzed for loss of *PURA* and *PURB*

Patient	*S/A*	*Diagnosis*	*Karyotype*
22	M/70	MDS	46,XY,**del(5)(q21q33),–7**,+mar[4]/45,XY,t(3;16)(p13;q22),**del(5)(q21q33),–7** [11]/46,XY [6]
23	M/74	MDS	46,XY,–3,**del(5)(q14q35),–7**,add(7)(p22),der(11)t(3;11)(p12;p14),+mar1,+mar2 [30]/46,XY [1]
24	M/20	t-MDS	44,XY,t(2;6)(q37;q21),**del(5)(q15q35),–7**,del(9)(q21),–17,add(17)(p13) [13]/46,XY [2]
25	F/72	t-MDS	45,XX,der(3)t(3;?)(q11;?),**del(5)(q21q34),–7**, add(11)(q25),del(13)(q13) [30]
26	F/68	t-MDS	45,XX,–3,–**5,**–**7**,–18,+mar1,+mar2,+mar3 [21]/44,XX,–3,–**5**,–17,–18,+mar2,+mar3 [4]/46,XX [1]
27	F/44	MDS	46,XX,r(4),**del(5)(q12q34),–7**,–15,add(17)(p13),–18,add(21)(q22),+mar1,+mar2,+mar3 [18]/45,XX,–4, **del(5)(q12q34),–7**,–15,add(17)(p13),–18,add(21)(q22),+mar1,+mar2,+mar3 [7]/46,XX [5]
28	F/83	MDS	43,XX,der(3)t(3;8)(q21;?),–**5,t(7;16)(p11;q11)**,der(12)t(12;17)(p12;q24),–15,–17,der(20)t(20;?),+mar [14]/45,XX,**t(5;11)(q14q33;q24),t(7;16)(p11;q11)**,der(12)t(12;17)(p12;q24),–15, –17,+mar [16]
29	M/24	MDS	45,XY,**der(5)t(5;?)(q11;?),–7**,del(12)(p13),r(13) [11]/44,XY,**der(5)t(5;?)(q11;?)**,der(6)t(6;12)(p25;q12),–**7**,–12,r(13) [5]/46,XY [6]
30	M/76	MDS	46,XY,**del(7)(p12p15)** [28]

S, sex; A, age; M, male; F, female; MDS, myelodysplastic syndrome; t-MDS, therapy-related MDS.
Bold indicates abnormality of chromosomes 5 and 7.

Fluorescence in situ hybridization and detection

FISH was performed as previously described.[36] Each slide was hybridized with 150 ng of labeled BAC DNA and/or 1 l of each probe from Vysis as recommended by the manufacturer. DIG and biotin-labeled probes were detected with anti-DIG-FITC, anti-DIG-rhodamine, or avidin-FITC (Vector Laboratories, Burlingame, CA, USA). DNA was identified by 4,6-diamidino-2-phenyllindole-dihydrochloride (DAPI) staining. For interphase FISH (I-FISH) 200–500 nuclei were scored per sample. The metaphase and interphase cells were viewed and imaged on an Axioskop epifluorescent microscope (Zeiss, Germany) equipped with a cooled CCD camera and CytoVision software (Applied Imaging, Pittsburgh, PA, USA).

Polymerase chain reaction analysis with sequence-specific and microsatellite markers

The primers used to amplify a 188 bp sequence of *PURA* were: 5′-GAT GTG GGC TCC AAC AAG TAC GGC-3′ and 5′-GTG AAG CTG CTC ACA GGC AGC CCG-3′. Primer sets for polymorphic marker loci were obtained from Research Genetics, or GeneLink (Thornwood, NY, USA) synthesized oligonucleotides with primer sequences obtained from the Genome Database (http://gdbwww.gdb.org) or the Lawrence Berkeley National Laboratory (LBNL) Human Genome Center human STS table (http://www-hgc.lbl.gov/sts.html). Reactions contained 10 ng of BAC DNA template, as well as the following: 500 ng of each primer, 10× polymerase buffer (Roche Molecular Biochemicals) to final concentration of 1×, 200 M of each dNTP (GIBCO BRL, Grand Island, NY, USA), and 0.5 U of Taq polymerase (Roche Molecular Biochemicals). The thermocycling parameters were as follows: initial denaturing at 94°C for 4 min; 15 cycles at 94°C (45 s), 55–62°C (30 s), and 72°C (2 min); and final extension at 72°C for 4 min. Then, 5 l of each PCR reaction was separated on a 1.8% agarose gel and stained with ethidium bromide.

Results

Deletion of the PURA gene is strictly correlated with deletion of EGR.1 at 5q31

Dual-color FISH analyses on 50 normal metaphase spreads reveal that the *PURA* probe (labeled BAC 153O15 DNA) hybridizes telomeric to the *EGR-1* probe and centromeric to the labeled STS-BAC pair D5S658 on chromosome 5, band region 5q31 (Figure 1a). FISH analysis with the *PURA* probe on 30 samples from healthy bone marrow transplant donors revealed the frequency of two fluorescent hybridization signals to be 97.8 ± 1.0% (s.d.) of the cells analyzed.

FISH analysis has been performed to determine the number of *PURA* copies and whether or not a *PURA* allele is deleted in the abnormal cells of patients with myeloid disorders and abnormalities of chromosome 5. I-FISH analysis with the *PURA* and *EGR-1* probes on samples from 24 patients identified as having del(5q) or –5 by conventional cytogenetics (Table 1) revealed 22 of 24 (91.7%) had one copy of the *PURA* and *EGR-1* genes; 17 of the 19 patients (89.5%) identified as having del(5q) and in all five patients having –5. An example of dual-color FISH with these probes on cells from a patient with del(5q) is shown in Figure 1b. None of the samples studied with the *PURA* and *EGR-1* probes identified a deletion of one gene and not the other.

Two of the 19 patients with del(5q), Nos 4 and 5, had been identified by conventional cytogentics to have del(5)(q14q32) and del(5)(q12q34), respectively, but I-FISH analysis showed that 98% of the cells in each sample had two copies of *PURA* and *EGR-1*. Additional FISH studies on previously G-banded chromosomes from patient No. 4 showed that the *PURA* and *EGR-1* loci were retained on the deleted chromosome 5 (data not shown). This indicated that both chromosomal breakpoints had been centromeric to these genes and were (q12q23). FISH analysis on G-banded chromosomes from patient No. 5 revealed that a region of 5q31, including the *PURA* gene, was inserted into chromosome 9 (Figure 2c).

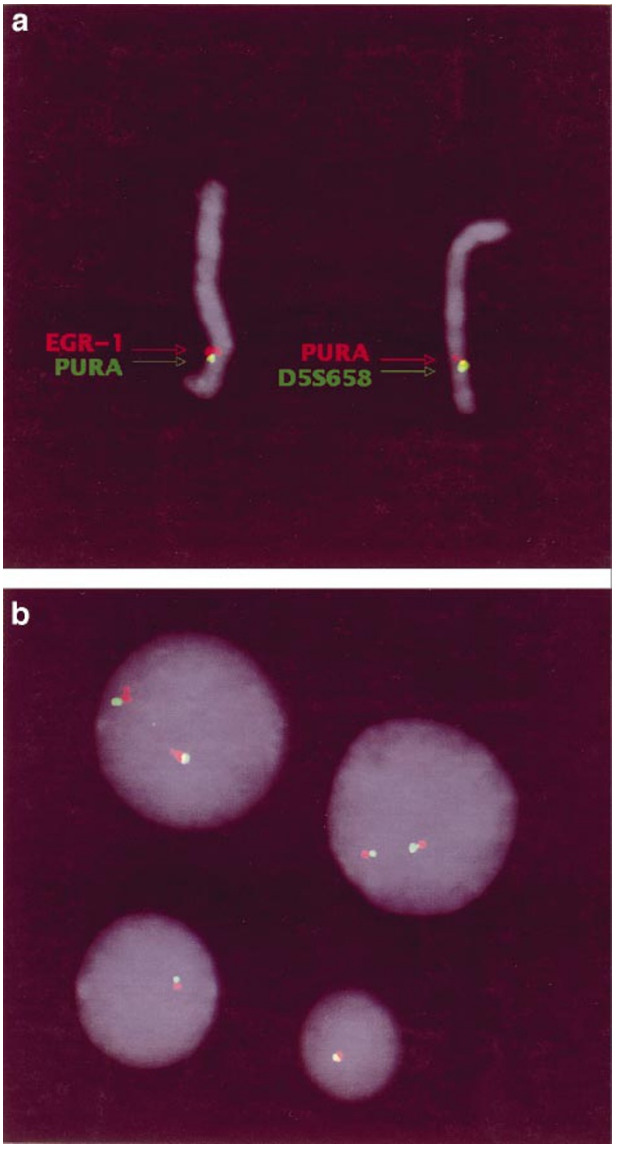

Figure 1 BAC DNA containing *PURA* used in FISH analysis of chromosome 5, band region 5q31. (a) Chromosomal localization of *PURA* probe, BAC 153O15 DNA, by FISH on metaphase spreads of peripheral blood mononuclear cells from a normal individual. Shown is an individual chromosome 5 from two separate dual-color FISH experiments. Left: *PURA* (green) is telomeric to *EGR-1* (red). Right: *PURA* (red) is centromeric to D5S658 (green) in band region q31.1. B. Shown are four interphase cells from patient No. 3 that were hybridized with the *PURA* (green) and *EGR-1* (red) probes. The two upper nuclei contain two copies of each gene, and the two lower nuclei have only one copy of each gene. DNA counterstain is DAPI.

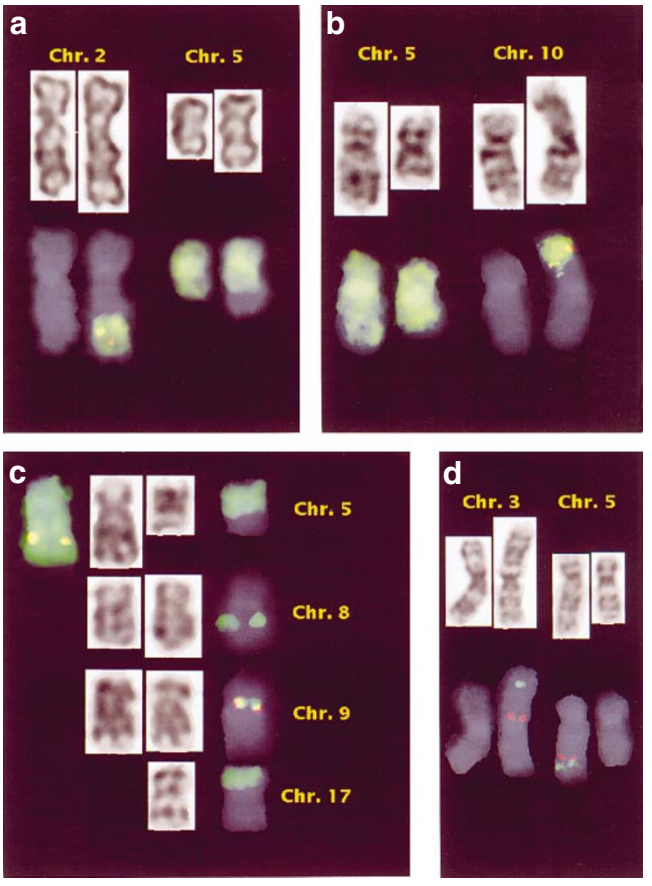

Figure 2 FISH with the *PURA* probe reveals 5q rearrangements. Chromosomes shown in panels a, b and c were hybridized with *PURA* (red) and WCP 5 (green) probes. (a) G-banded chromosomes 2 and 5 from patient No. 15 are shown in the top row. FISH studies shown in the bottom row reveal t(2;5)(q37;q14), including the translocation of one *PURA* of allele to chromosome 2. (b) G-banded chromosomes 5 and 10 from patient No. 16 are shown in the top row. FISH studies shown in the bottom row reveal t(5;10)(q21;p14) that had been interpreted as t(10;17). C. Patient No. 5 had del(5)(q12q34) as well as nine other abnormalities identified by conventional cytogenetics. G-banded chromosomes 5, 8, 9 and 17 are shown. As seen in the third row, FISH studies have identified the insertion of *PURA* (red) into chromosome 9, ins(9;5)(?p1;q31). Other rearrangements involving chromosome 5 that have been identified by FISH are der(5), ins(8;5)(q22;?), and der(17)t(5;17)(?;p11). (d) Previously G-banded chromosomes from patient No. 19 were hybridized with *PURA* (red) and D5S487 (green) probes. G-banded chromosomes 3 and 5 are shown in the top row. One hybridization signal for each probe is translocated to chromosome 3, but a large distance between the labeled loci is observed on the translocated 5 compared to the normal chromosome 5. The proximal breakpoint of the inverted region of chromosome 5 is q13, and the distal breakpoint is in band region q31 just telomeric to the *PURA* locus.

In a separate experiment (data not shown) it was shown that one *EGR-1* allele was also inserted into the chromosome 9 near the *PURA* locus. FISH experiments on the sample from patient No. 5 illustrate that even when a rearrangement involves a very small region of 5q31, the *PURA* and *EGR-1* genes are simultaneously involved. Furthermore, clarification of the karyotypes of these two patients by FISH reveals that in all 17 cases displaying del(5q), including band region 5q31, *PURA* is deleted.

Another two of the 19 patients with del(5q), Nos 15 and 16, had been identified by conventional cytogenetics as having interstitial deletions in both chromosomes 5 including band region 5q31, suggesting potential homozygous deletion of *PURA*. However, one hybridization signal was observed by I-FISH with *PURA* in cells from patients Nos 15 and 16. Hybridization of the *PURA* probe and WCP 5 on previously G-banded chromosomes from these two patients identifies translocations of 5q (Figure 2a and b). In both of these cases, and in case No. 14 (data not shown), FISH studies thus identify a deletion of one *PURA* allele and a rearrangement of the second allele.

In order to determine whether or not *PURA* and *EGR-1* loci are deleted as part of a microdeletion, FISH studies were per-

formed on samples from 30 patients diagnosed with MDS or AML and identified as having normal karyotypes. In all 30 cases analyzed, 97.9 ± 1.0% (s.d.) of the cells had two copies of each gene.

Loss of PURA frequently accompanies 5q translocations

Seven of the 30 selected patients with abnormal karyotypes had been identified by conventional cytogenetics as having translocations involving 5q. Four of the seven patients (Nos 18, 20, 21 and 29) were found by FISH studies to have a loss of a *PURA* locus. In the three cases with unbalanced translocations (Nos 20, 21 and 29), FISH studies identified the deletion of the long arms of chromosome 5 from band region q11 to qter (data not shown). After FISH analysis had revealed the loss of one *PURA* allele in the cells of patient No. 18, the karyotype was reevaluated. A new karyotype was determined: 46,XX,der(5)del(5)(q11)t(1;5)(p13;q11),del(11)(q14).

One of the seven patients (No. 19) identified with a translocation of 5q was shown by FISH to have an inversion of 5q in the translocated segment. This inversion is revealed by hybridization of both the *PURA* probe and labeled STS-BAC pair D5S487, localized to band region 5q32, to metaphase spreads (Figure 2d). The hybridization signals from the probes indicate that a distal chromosomal breakpoint exists between these loci, in addition to the proximal breakpoint at q13, and that there is an inversion accompanying the rearrangement of chromosome 5. It is important to note the close proximity of the breakpoint to the *PURA* locus in this event, and those shown in Figure 2b and c, as well as the fact that the break occurs telomeric to *PURA*.

High-resolution physical mapping locates PURA near the STS D5S1867 and D5S352 loci that is approximately 1.1 Mb telomeric to the EGR-1 gene

High-resolution physical mapping of the *PURA* gene was performed using PCR techniques as well as FISH. The *PURA* gene was shown to be telomeric to the *EGR-1* gene and centromeric to the STS D5S658 locus on chromosome 5 (Figure 1a). DNA from two overlapping BAC clones containing *PURA* (153O15 and 507K3) was used as the template for PCR amplification with primer sets for several STS markers located between these two loci. Amplification of the *PURA* gene sequence and the genomic marker loci STS D5S1867, bac51304S, and bac51305T was observed for BAC 507K3 DNA (Figure 3).

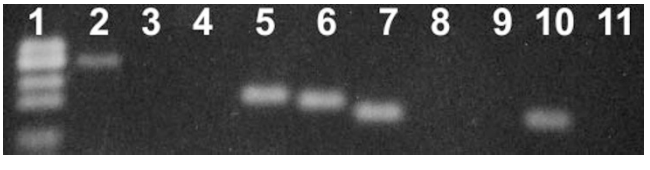

Figure 3 PCR amplification of BAC 507K3 DNA identifies location of *PURA* near D5S1867. Shown is an ethidium bromide-stained 1.8% agarose gel. Lane 1 contains ×174 DNA digested with *Hae*III. The bands are, from the top, 281, 271, 243 and 194 bp, respectively. Five l of solution were loaded from PCR reactions on BAC 507K3 DNA template with the following primer pairs: lane 2, D5S352; lane 3, IB372; lane 4, AFMb290yc9; lane 5, bac51304S; lane 6, D5S1867; lane 7, bac51305T; lane 8, bac5365T; lane 9, bac51304T; lane 10, PSYA primers for *PURA*; lane 11, no DNA and PSYA primers. Lanes 3 to 9 are in order (left to right) from centromere to telomere on 5q (LBNL Human Genome Center maps).

From these results, PCR amplification of BAC 153O15 DNA (data not shown), and the sequence of a BAC clone including marker locus bac151304S (GB accession No. AC005575), we have mapped the *PURA* gene within 100 kb of the STS D5S1867 locus between bac15304S and bac5365T loci. Amplification was also observed using primers for an orphan STS locus, D5S352 (Figure 3) locating it within 100 kb of the *PURA* gene. The orientation of *PURA* and D5S352 with respect to each other and to the D5S1867 locus remains to be determined. A map of the *PURA* locus at 5q31 is presented in Figure 4a.

Identification and mapping of the human PURB gene at 7p13

The functional importance of Pur protein dimerization led to the investigation of the gene encoding the human Pur protein. We have identified the full-length human *PURB* in a high-throughput human genomic sequence entered with GenBank (clone D50673M15; GB accession No. AC004854). The coding region of *PURB* is 939 bp with a single open reading frame of 312 amino acids. The predicted human Pur amino acid sequence was compared to the derived amino acid sequences of human Pur [19] and mouse Pur [20] proteins. The human Pur has 65% identity and 71% similarity to human Pur protein; and it has 94% identity to the mouse Pur protein.

We used STS marker sequence D7S478, located 7344 bp

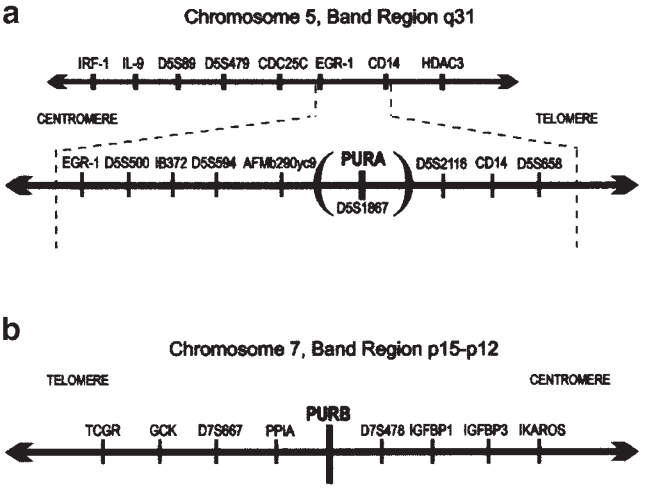

Figure 4 Locations of *PURA* and *PURB* on chromosomes 5 and 7. (a) Illustrated is the location of the *PURA* gene in relation to other genes and markers that have been mapped to band region 5q31.1. The order of the markers shown is adopted from bacmaps at the LBNL Human Genome Center. Other genes mapped to chromosome 5 band region q31 encode for proteins that include: interferon regulatory factor 1, IRF1;[12] interleukin-9, IL-9; a CDC2 phosphatase, CDC25C; early growth response-1 zinc-finger protein, EGR-1;[46] myelomonocytic differentiation antigen, CD14;[13] and a histone deacetylase, HDAC3.[47] (b) Illustrated is the location of the *PURB* gene in relation to other genes and markers that have been mapped to 7p. The order of the genetic markers and genes shown is adopted from The Genethon Genetic Map at the National Human Genome Research Institute (www.genome.nhgri.nih.gov/chr7/YAC__STS/CONTIGE). Other genes mapped to 7p encode proteins that include: T cell antigen receptor gamma polypeptide TCRG;[48] glukokinase, GCK;[49] intracellular cyclosporin A receptor, cyclophilin A (PPIA);[50] insulin-like growth factor binding proteins, IGFBP-1[51] and IGFBP-3;[52] and lymphocyte-restricted zinc finger DNA-binding protein, IKAROS[53]

from the *PURB* sequence, to identify a BAC clone for use as a FISH probe (Figure 4b). FISH mapping with labeled BAC GS025I23 DNA, the *PURB* probe, localized this DNA to chromosome 7, band region p13 (Figure 5a). FISH studies with the *PURB* probe on a specimen from a patient diagnosed with MDS (No. 30) that had an interstitial deletion of the short arms of chromosome 7, del(7)(p12p15), as the sole abnormality demonstrated that only one copy of *PURB* was present in the abnormal cells of this patient. A representative metaphase spread from the sample illustrates the loss of one *PURB* allele (Figure 5b).

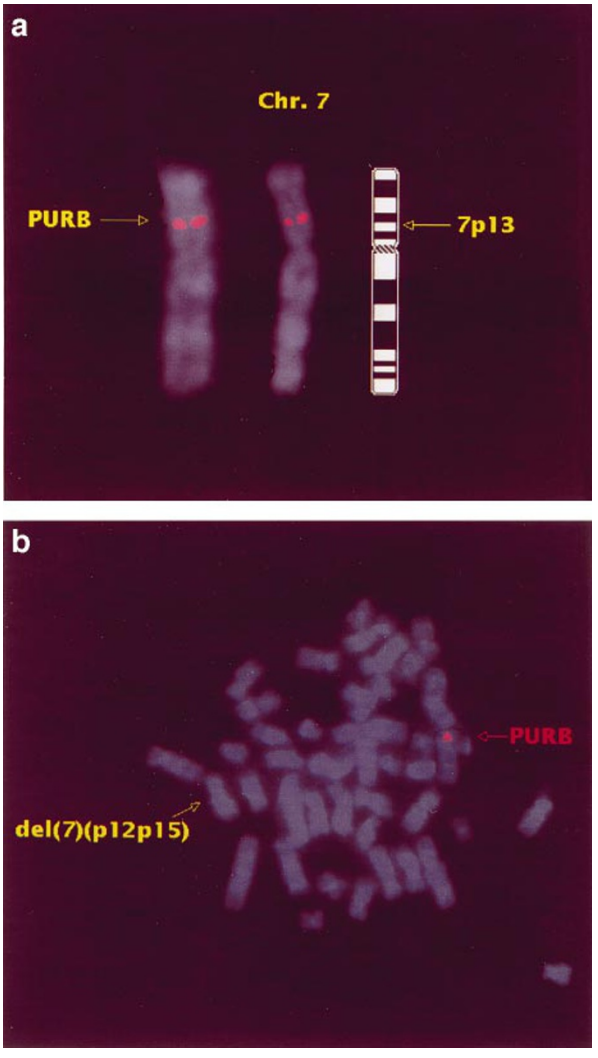

Figure 5 FISH studies localize *PURB* to chromosome 7, band region p13, and show the loss of the *PURB* locus in a MDS patient with del(7)(p12p15). (a) Labeled *PURB* probe, BAC GS025I23 DNA, hybridized to metaphase spreads of peripheral blood mononuclear cells from a normal donor. Hybridization signals (red) are located on chromosome 7, band region p13, indicated on the chromosome 7 ideogram with the yellow arrow. (b) A representative metaphase spread from FISH studies with the *PURB* probe (red) on a sample from patient No. 30. The probe hybridizes to only one locus. The yellow arrow indicates the chromosome del(7)(p12p15) that is identified by inversion of the DAPI counterstain (CytoVision, Applied Imaging) and that had been previously identified by conventional cytogenetics.

Simultaneous loss of PURA and PURB genes

Simultaneous hemizygous deletions of *PURA* and *PURB* were investigated in eight MDS patients. In specimens from six patients with MDS in which del(5q)/–5 and –7 had been identified by conventional cytogenetics, monosomy of *PURA* and *PURB* was observed in interphase cells from all six. Illustrated in Figure 6a is a metaphase spread from one of the six patients (No. 25), which shows the presence of one copy of *PURA* and one copy of *PURB*. A seventh patient (No. 29) had a der(5) as well as –7 identified by conventional cytogenetics, and monosomy *PURA* and *PURB* was observed by FISH. In an eighth patient (No. 28) cytogenetics had identified –5 in addition to a rearrangement of 7p. FISH analysis was done in order to determine if this rearrangement of 7p had resulted in loss of

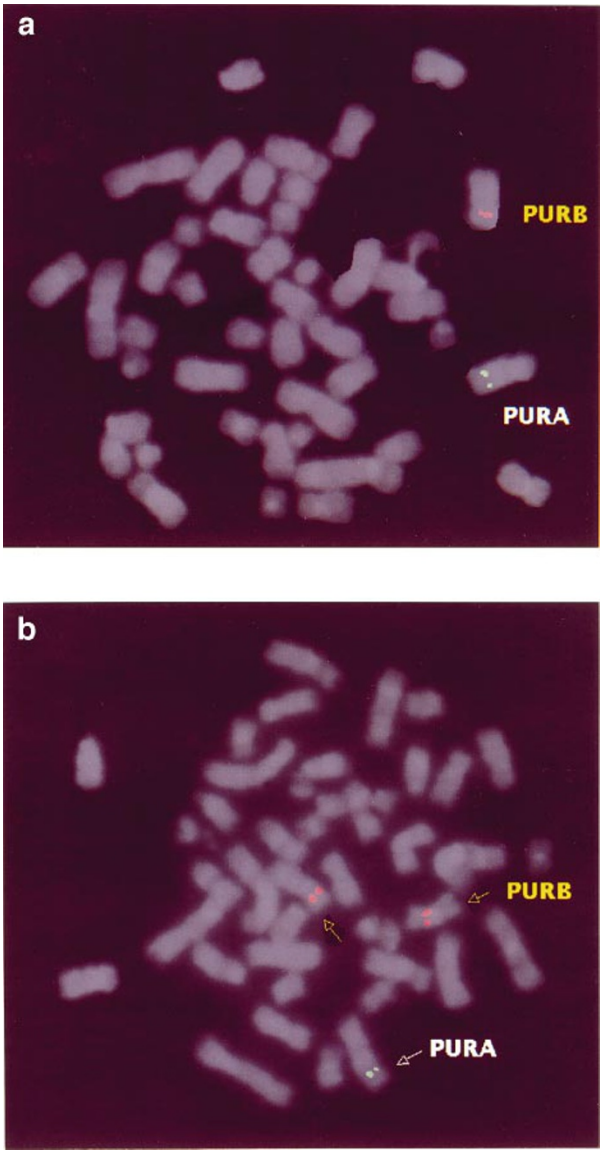

Figure 6 Simultaneous loss or rearrangement of *PURA* and *PURB*. Representative metaphase spreads from two patients (Nos 25 and 28) hybridized with *PURA* (green) and *PURB* (red) probes are shown. (a) Hybridization of only one *PURA* and one *PURB* allele is observed in this cell from patient No. 25. (b) Hybridization of only one *PURA* allele and rearrangement of one *PURB* allele is observed in this cell from patient No. 28.

PURB. The I-FISH studies showed that the abnormal cells had *PURA* monosomy and *PURB* disomy (Figure 6b).

Discussion

In all of the specimens examined in which a bona fide deletion at 5q31 was present, each deletion included the *PURA* and *EGR-1* loci. The data presented indicate that *PURA* and *EGR-1* are approximately 1.1 Mb apart with *PURA* telomeric (Figure 4). The high frequency of deletion of *PURA* in patients with 5q abnormalities and the strict correlation of deletions of *PURA* and *EGR-1* indicate that the minimally deleted regions reported to be between polymorphic markers D5S479 and D5S500[5] and between D5S500 and D5S594[11] most likely extend telomeric to the *PURA* locus. The borders of these regions are approximations based on LOH of loci that are spaced in most cases 50 kb apart on small groups of specimens. Three cases analyzed in this study with *PURA* were identified as having interstitial deletions with distal breakpoints in band region 5q31. Our data indicate that the minimally deleted region of 5q31 deleted in MDS and AML extends telomeric at least to *PURA*. FISH studies performed on samples from patients diagnosed with MDS or AML and identified as having normal karyotypes indicated that microdeletions of these loci did not occur in these patients, and if microdeletions do occur they are rare events.

Simultaneous loss of *PURA* and *PURB* loci is most interesting since it is the first instance of such a phenomenon involving genes encoding proteins that function as a heterodimer. Concurrent loss of one *PURA* and one *PURB* allele was observed in all six of the studied patients and in one additional case with der(5) and –7. Other investigators have reported that as many as 22% of patients with t-MDS/t-AML have co-segregating abnormalities of chromosomes 5 and 7.[4] Also associated with these concurrent abnormalities is a poor prognosis and short survival.[37,38] From January 1986 to September 1999, 862 patients diagnosed with MDS were evaluated for cytogenetic abnormalities in this laboratory. Abnormal karyotypes were identified in 289 (33.5%) of the cases. Monosomy 5/del(5q) was observed in 29.1%, –7/del(7q) in 22.8%, –7 in 14.2%, and del(7p) in 1.0% of the patients with abnormal karyotypes. Importantly, occurrence of –5/del(5q) and –7/del(7q) was observed in 11.0% of the 289 patients with abnormal karyotypes, 5.9% of them having –5/del(5q) and –7 simultaneously. If the present results can be extrapolated to this entire population, the claim can be made that virtually all patients with –5/del(5q) and –7 are hemizygous for *PURA* and *PURB* loci. That figure of 5.9% represents a minimum for patients with simultaneous deletions of *PURA* and *PURB*, as patients showing del(7)(p13) and/or translocations of 5q which have loss of *PURA* are not included. The figure of 5.9% is nearly 1.5-fold higher than the 4% expected on the basis of the independent occurrence of –5/del(5q) and –7. Of course, the same excess of expectation would apply to any two genes located at band region 5q31 and chromosome 7. It is notable, however, that no two other genes, encoding closely related family members, have yet been identified in these regions.

In MDS deletions of 7q are more frequently reported than deletions in 7p, and it is possible that one or more genes on 7q are tumor suppressors or otherwise involved in myeloid pathogenesis. Nonetheless, deletions in 7p do occur, and it is likely that genes on 7p are involved in progression to AML in a fashion distinct from those on 7q. In a study of 716 patients with AML it was found that 4.0% had deletions of 7q while 7.7% had monosomy 7.[39] This preference for monosomy suggests that more than one gene on chromosome 7, including gene(s) on 7p, may be involved in progression to AML. Note that in this study the only cytogenetic abnormality observed in patient No. 30 is del(7)(p12p15), which includes the *PURB* locus.

Are concurrent deletions at 5q and 7p involved in a progression from MDS to AML? A statistical analysis of this question is intriguing. As discussed above, whereas concurrent del(5q)/–5 and –7 is expected in 4% of MDS patients with karyotypic abnormalities, they are seen in 5.9% of cases. In AML, and primarily *de novo* AML, according to a study of 716 patients, del(5q)/–5 is seen in 12.1%, and –7 in 7.7%.[39] Whereas expected in 1% of the cases, simultaneous del(5q)/–5 and –7 is seen in 5.6% of cases. The percentages would be reflected in deletions of *PURA* and *PURB* loci. These figures suggest that progression from MDS to AML is strongly preferential for concomitant del(5q)/–5 and –7 relative to either one alone. In interpreting these figures it must be kept in mind that deletions in chromosomes 5 or 7 are frequently debilitating and that many patients with them may not progress to AML because they do not survive MDS. Deletions in chromosomes 5 or 7 also indicate a poor response to therapy for AML.[40] AML patients with abnormalities in chromosomes 5 or 7 are least likely to achieve complete remission and display the shortest median survival time. In a study of 66 long-term survivors of AML four of 11 patients with del(7q) were alive after 10 years, whereas only one of 24 patients with –7 survived 10 years.[37] This comparison suggests that gene(s) on 7p may be important for long-term survival.

At this point we have not detected mutations in the *PURA* gene coding sequence, using PCR and SSCP, in peripheral blood cells from patients with deletions of 5q31 and MDS (data not shown). DNA from three patients (Nos 3, 8 and 18) was examined for the presence of constitutional mutations using a PCR-based single-stranded conformational polymorphism (SSCP) analysis.[41] These data indicate that in these three patients there was no germ line transmission of an aberration in *PURA*. Clearly, many patients must be studied, and extensive sequencing must be performed, particularly on DNA samples from abnormal bone marrow cells and including the gene regulatory region, to completely rule out *PURA* as a tumor suppressor gene. At this point, however, we have no evidence that *PURA* is a prototypic tumor suppressor gene.

The function of the human Pur protein has not been extensively studied, but the mouse Pur protein, which is 94% homologous to the human protein, has been shown to be important in gene transcription. The lack of nucleotide sequence homology outside of *PURB* with chromosome 5, band region q31.1, supports the fact that the *PURB* sequence is not an evolutionarily recent gene duplication. Evidence has been reported that *PURA* is a member of a tightly conserved homology group (HG53) between human and mouse.[24] Investigation of the homology maps at the Online Mendelian Inheritance in Man (OMIM) site (http://www.ncbi.nlm.nih.gov/omim) reveals that *PURB* (Figure 4) to lie between *GCK* (MIM No. 138079) and *IGFBP-1* (MIM No. 146730) may be a member of the conserved homology group 74 (HG74). The genes in HG74 on human chromosome 7 are conserved with genes located on mouse chromosome 11. Functional conservation of human and mouse Pur is suggested by the fact that both proteins have complete homology in the three 'class I' DNA-binding domains.[20]

Four lines of evidence suggest that the Pur protein may

be involved in cell cycle checkpoint control. Firstly, the Pur protein has been shown to bind to the hypophosphorylated form of Rb in a cell cycle-dependent manner.[42] Secondly, Pur protein levels have been observed to change during the cell cycle.[43] Thirdly, high intracellular levels of Pur have been reported to arrest cell cycle progression.[44] Even modestly increased levels of Pur can reverse certain aspects of *ras* transformation of NIH3T3 cells (S Barr and EM Johnson, *J Cell Biochem* 2001, in press). Mutations in the Rb protein are rarely reported in myeloid disorders,[45] whereas deletions of *PUR* gene family members are relatively common. It is possible that Pur and Pur function in a pathway involving Rb leading to G1/S checkpoint control. Since deletions of *PURA* and *PURB* are frequently observed in MDS, loss of such control may be an early step in progression to leukemia.

Pur and Pur proteins are reported to function together as a heterodimer, and the properties of the homodimer and heterodimer of these proteins differ substantially in that Pur exerts a repressive effect that antagonizes a stimulatory effect of Pur on gene transcription.[30] Our results raise the question of whether Pur and Pur function may be subject to haploinsufficiency effects. In the absence of gene imprinting it is generally believed that most genes are regulated codominantly, with both alleles contributing to expression. A model invoking haploinsufficiency has been proposed for the gene encoding the CBFA2 transcription factor, one allele of which is mutated in family platelet disorder with predisposition to AML.[16] In that study nonsense mutations were observed that inactivate a protein encoded by one allele. Even though alterations in Pur or Pur levels may be virtually undetectable by immunoblotting, they could well alter a delicate balance involved in heterodimer formation. Simultaneous hemizygosity of *PURA* and *PURB* could thus affect the balance of homo- and heterodimers of the Pur and/or Pur proteins. To address this would necessitate analysis of Pur and Pur levels in the cells of patients' marrow specifically possessing deletions of one allele. This is beyond the scope of the present paper. The fates of mice genetically altered to possess $PURA^{-/-}$ or $PURA^{+/-}$ genotypes are currently being evaluated to shed additional light on this prospect.

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