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Chromosome Band 1p36 Contains a Putative Tumor Suppressor Gene Important in the Evolution of Chronic Myelocytic Leukemia

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Chronic myelocytic leukemia (CML) is a common neoplasm of hematopoietic pluripotent stem cells. Although the evolution from chronic phase to blast crisis (BC) in CML patients is an inevitable clinical feature, little is understood about the mechanisms responsible for the transformation. We have previously performed allelotype analysis in CML BC and have detected frequent loss of heterozygosity (LOH) on the short arm of chromosome 1. To know the common region of LOH where a putative tumor suppressor gene may reside, deletional mapping was performed using 33 microsatellite markers spanning chromosome 1 in 30 patients with CML BC (21 myeloid and 9 lymphoid). DNA was extracted from slides of bone marrow smears or from bone marrow mononuclear cells. In each patient, DNA from chronic phase was analyzed alongside DNA from either their BC or accelerated phase. Allelic loss on 1p was observed in 14 of the 30 individuals (47%): 10 of the 21 myeloid and 4 of the 9 lymphoid BC cases. Serial cytogenetic information was available in 10 cases with LOH on 1p; interestingly, deletions in this region were not detected. Two samples showed LOH at all informative loci on 1p, whereas the other 12 samples showed LOH on at least one but not all loci on 1p. The common region of LOH resided proximal to *D1S508* and distal to *D1S507* (1p36). Our results suggest that a tumor suppressor gene that frequently plays an important role in the evolution to BC resides on 1p36 in CML.

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SEVERAL LINES OF evidence have shown that inactivation of tumor suppressor genes is intimately associated with tumorigenesis in a wide variety of human tumors.[1] The two-mutation hypothesis suggested that both alleles of a tumor suppressor gene are inactivated in tumors.[2] In fact, such inactivation of a tumor suppressor gene has been commonly caused by a mutation of one allele accompanied by loss of the second allele. To date, loss of heterozygosity (LOH) has been reported to occur on various chromosomal regions in diverse tumor types. Furthermore, several tumor suppressor genes have been identified and characterized from some of these regions showing frequent LOH in tumors.

Chronic myelocytic leukemia (CML) is a common hematologic neoplasm of pluripotent hematopoietic stem cells. More than 90% of CML cases show the characteristic Philadelphia chromosome (Ph), which results in the fusion of sequences of the *ABL* gene from chromosome 9 with sequences of the *BCR* gene from chromosome 22.[3,4] In the absence of effective therapy, the progression from chronic phase to blast crisis (BC) in CML patients is an inevitable clinical feature and results in a fatal process. Abnormalities of the tumor suppressor genes, such as mutations of the *p53* gene, absence of RB protein, and homozygous deletions of the $p16^{INK4a}$ gene, have been reported to occur during this process in a subset of CML cases.[5-8] Abnormalities of other tumor suppressor genes may also lead to blastic transformation of CML.

Allelotype analysis is a powerful method to identify the regions that have LOH.[9-11] We have previously performed allelotype analysis in 30 patients with CML BC and have detected frequent LOH at two loci on the short arm of chromosome 1.[12] To define a common region of allelic loss that may harbor putative tumor suppressor gene(s), we performed deletional mapping of chromosome arm 1p in CML BC.

MATERIALS AND METHODS

Samples. Paired bone marrow samples of chronic phase and either BC or accelerated phase (AP) were obtained from each patient with CML. Clinical information was available for all 30 patients as shown in Table 1. Seventeen patients were male and 13 patients were female. Twenty-one patients had myeloid crisis and 9 had lymphoid crisis. Twenty-six BC and 4 AP samples were obtained from the 30 patients. The mean percentage of blasts for the BC or AP samples was 54.4% (range, 10% to 90%). Cytogenetic data were available for 29 patients.

Extraction of DNA. DNA was extracted from slides of bone marrow smears derived from each patient. Cells were stripped off slides with disposable scalpels, washed once with xylene, washed twice with absolute ethanol, and were lysed with 50 mmol/L Tris-HCL (pH 8.5), 1 mmol/L EDTA, 0.5% Tween 20, and proteinase K in a final concentration of 100 µg/mL at 37°C overnight. DNA was extracted twice with phenol and once with chloroform, and was precipitated in ethanol. The DNA pellets were dissolved in 40 µL of TE (10 mmol/L Tris-HCL pH 7.4, 1 mmol/L EDTA). DNA was extracted from bone marrow mononuclear cells from 7 patients after obtaining their informed consent (CML nos. 5 through 7, CML nos. 23 through 26).[13]

LOH analysis. Polymerase chain reaction (PCR)-amplification of microsatellite sequences was used to determine LOH. Primers for microsatellite sequences were obtained from Research Genetics (Huntsville, AL).[14,15] Each PCR reaction contained 10 ng of DNA, 5 pmole of each primer, 1 nmole of each dNTP (Pharmacia, Stockholm, Sweden), 0.3 U of Taq DNA polymerase (GIBCO-BRL, Gaithersburg, MD), 2 µCi of [α-^{32}P]dCTP (ICN, Irvine, CA) in 10 µL of the specified buffer with 1.5 mmol/L $MgCl_2$. Thirty-two cycles of denaturing for 40 seconds

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Table 1. Clinical Characteristics of CML Patients

Sample	Age	Sex	Phenotype	Blast (%)*	Karyotype†
CML1	48	M	Myeloid	20	46,XY,t(9;22)(q34:q11)
CML2	69	F	Myeloid	85	46,XX,t(9;22)(q34:q11)
CML3	39	F	Myeloid	50	NA
CML4	33	M	Myeloid	30	46,XY,t(9;22)(q34:q11)
CML5	45	M	Myeloid	10	46,XY,t(9;22)(q34:q11)
CML6	45	M	Myeloid	65	47,XY,t(9;22)(q34:q11),+der(22)t(9;22)(q34:q11)
CML7	19	M	Myeloid	30	47,XY,t(9;22)(q34:q11),+der(22)t(9;22)(q34:q11)
CML8	33	F	Myeloid	21	NA
CML9	38	M	Myeloid	NA	46,XY,der(3)t(3;7)(p13;p13),der(7)t(3;7)(p23;p13),t(9;22)(q34:q11)
CML10	39	F	Myeloid	16	46,XX,del(2)(q?),t(9;22)(q34:q11),add(11)(q?),add(15)(q?)
CML11	60	F	Myeloid	90	46,XX,t(9;22)(q34:q11)
CML12	60	M	Myeloid	75	46,XY
CML13	52	M	Myeloid	70	45,X,−Y,t(9;22)(q34:q11)
CML14	50	M	Myeloid	60	46,XY
CML15	73	M	Myeloid	57	46,XY,t(9;22)(q34:q11)
CML16	52	M	Myeloid	66	48,XY,+8,t(9;22)(q34:q11),+der(22)t(9;22)(q34:q11)
CML17	52	F	Myeloid	58	46,XX,t(9;22)(q34:q11)
CML18	61	F	Myeloid	64	46,XX,t(9;22)(q34:q11)
CML19	57	F	Myeloid	72	46,XX,t(1;21)(q32;q21),t(9;22)(q34:q11)
CML20	50	M	Myeloid	70	46,XY,t(9;22)(q34:q11)
CML21	53	F	Myeloid	90	46,XX,t(9;22)(q34:q11)
CML22	62	M	Lymphoid	40	NA
CML23	38	M	Lymphoid	82	47,XY,+?der(1)t(1;20)(q21;q11.2)del(1)(p11),−9,t(9;22)(q34:q11),+18, der(20)t(1;20)(q21;q11.2)
CML24	38	F	Lymphoid	60	47,XX,t(9;22)(q34:q11),+i(17)(q10)
CML25	31	M	Lymphoid	90	46,XY,t(9;22)(q34:q11)
CML26	34	F	Lymphoid	10	46,XX,t(9;22)(q34:q11)
CML27	48	M	Lymphoid	65	46,XY,t(9;22)(q34:q11)
CML28	60	M	Lymphoid	40	46,XY,t(9;22)(q34:q11)
CML29	38	F	Lymphoid	45	46,XX,t(9;22)(q34:q11)
CML30	52	F	Lymphoid	46	46,XX,t(9;22)(q34:q11)

Abbreviations: M, male; F, female; NA, not available.

*Blast (%) at BC or AP.

†Karyotype at BC or AP.

All but two individuals (CML nos. 12 and 14) had Ph chromosome at diagnosis. Rearrangement of the *BCR* gene was detected at diagnosis by Southern blot analysis for CML nos. 12 and 14. Four samples were from AP patients (CML nos. 1, 5, 10, and 26).

at 94°C, annealing for 30 seconds at 55°C, and extending for 35 seconds at 72°C were performed in a Programmable Thermal Controller (MJ Research Inc, Watertown, MA). After amplification, PCR samples were diluted in the loading buffer containing 20 mmol/L EDTA, 96% formamide, 0.05% of both bromophenol blue and xylene cyanol. The products were heated to 94°C for 5 minutes and chilled on ice. Three microliters of the dilutions was applied to a 5% to 6% polyacrylamide gel containing 8.3 mol/L urea and separated for 2 to 3 hours at 75 Watts. The gel was dried and subjected to autoradiography using Kodak (Eastman Kodak Company, Rochester, NY) XAR film at either room temperature or −80°C. LOH was scored in informative cases if a significant reduction (≥50%) in the signal of the allele from the BC or AP sample was visually noted in comparison to the corresponding allele from the chronic phase of the same individual. Where necessary, LOH was assessed by densitometry. In most samples showing LOH, PCR amplification and analysis were repeated to assure consistency. For several markers that showed frequent homozygosity in chronic-phase samples, we performed duplex semiquantitative PCR with the markers on 1p and markers from chromosome arms other than 1p.

RESULTS

We screened 30 paired CML samples for LOH with a panel of 33 highly informative microsatellite markers spanning chromosome 1. Thirty markers were located on 1p and 3 were on 1q. Each sample was analyzed at every marker. Some samples were not informative at several loci because of a shortage of DNA. All patients were informative at multiple loci on chromosome arm 1p. We performed duplex semiquantitative PCR at several loci that showed frequent homozygosity in chronic phase samples, and we did not find occult hemizygosity. Allelic loss on 1p was observed in 14 of the 30 cases of BC or AP (47%). The most frequent LOH (8 of 15 informative cases; 53%) was observed at the *D1S468* locus on chromosome band 1p36. Figure 1 shows examples of allelic loss. The lower allele was deleted at the *D1S468* locus in CML no. 11 (Fig 1A). Heterozygosity was retained at *D1S243* and *D1S199*. In Fig 1B, LOH was observed at *D1S228*, whereas heterozygosity was retained at *D1S243* and *D1S507* (CML no. 24). LOH was detected at *D1S2667*, whereas heterozygosity was retained at *D1S508* and *D1S199* (Fig 1C, CML no. 1). No instability was observed in this study.

Two of the 14 cases showed LOH at all informative loci on chromosome arm 1p. Twelve cases showed LOH on at least one marker on 1p but not at some other markers on this arm. Figure 2 shows the deletional map on chromosome 1 in the 14 cases of LOH. CML no. 1 showed allelic loss at *D1S2667* and *D1S2672*, and showed retention of heterozygosity at *D1S508* and *D1S436*. For CML no. 24, LOH was detected at *D1S450* and *D1S228*, and retention of heterozygosity was observed at *D1S2663* and *D1S507*. The consensus region of allelic loss was determined to reside proximal to *D1S508* and distal to *D1S507* (1p36). This region is estimated to encompass 19 centimorgans (cM) of genetic distance.

Cytogenetic information of BC as well as chronic phase was available for 10 of the 14 cases with LOH on 1p; however, deletions on 1p were not detected (Table 1). Clinical information was available for all 30 patients (Table 1). Allelic loss on 1p was observed in both myeloid (10 out of 21; 48%) and lymphoid (4 out of 9; 44%) BC.

DISCUSSION

The molecular genetic changes of CML have been well studied in the chronic phase. In contrast, little is known about the mechanisms responsible for BC. To understand the role of genetic changes in the evolution of CML, we performed deletional mapping of chromosome 1 using highly informative microsatellite markers. Frequent allelic loss was observed on chromosome arm 1p in 14 of the 30 cases of BC (47%).

The common region of allelic loss resided proximal to

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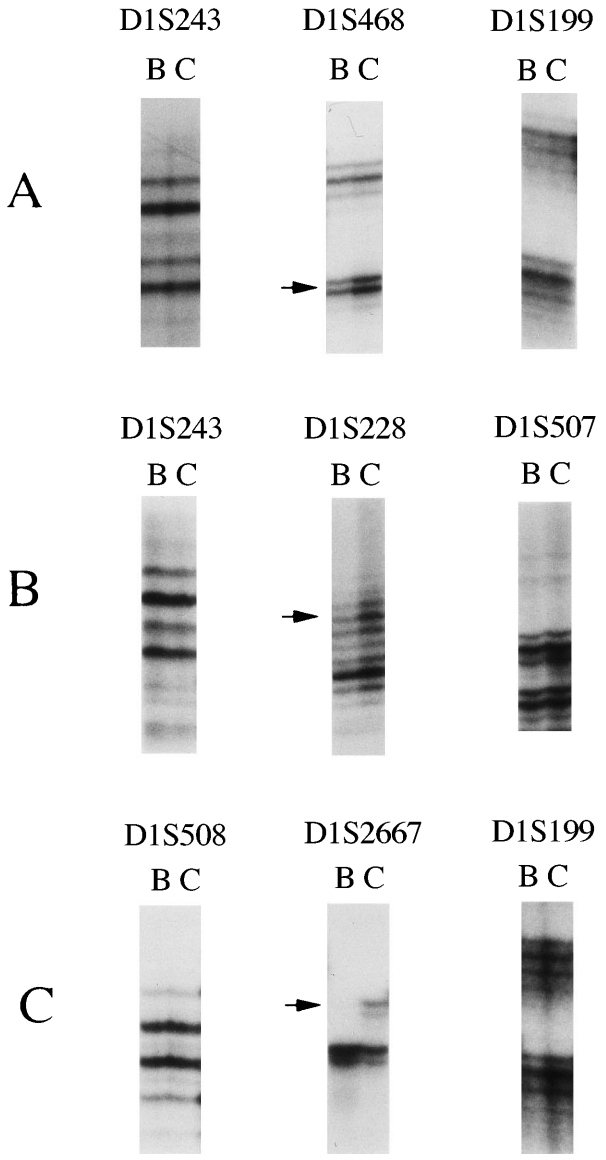

Fig 1. Loss of heterozygosity on chromosome arm 1p in CML. (A) LOH was detected on *D1S468* in CML no. 11. Heterozygosity was retained at *D1S243* and *D1S199*. (B) Allelic loss was observed at *D1S228*, whereas heterozygosity was retained at *D1S243* and *D1S507* (CML no. 24). (C) LOH was found at *D1S2667*, whereas heterozygosity was retained at *D1S508* and *D1S199* (CML no. 1). The locus symbol is shown on the top. Arrow indicates allele lost. B, blast crisis; C, chronic phase.

D1S508 and distal to *D1S507* (1p36). Frequent LOH has been reported in the same region in several types of tumors, such as neuroblastoma, colorectal, breast and hepatocellular carcinomas, parathyroid adenoma, and melanoma.[16-21] Allelic loss of 1p was associated with unfavorable outcome in neuroblastoma.[22] The common region of allelic loss in the present study partly overlapped with common regions of LOH in these solid tumors.[23] However, to date no altered tumor suppressor gene responsible for these tumors has been identified on chromosome region 1p36. Cloning of candidate gene(s) will define whether either a single or multiple tumor suppressor genes are clustered on 1p and are commonly involved in these types of tumors.

The $p18^{INK4c}$ gene is located on 1p32 and it is regarded as a tumor suppressor gene.[24] Homozygous deletions of the $p16^{INK4a}$ gene, which is a homolog of the $p18^{INK4c}$ gene, were reported in lymphoid BC of CML.[6] While the common region of LOH in the present study was more distal (1p36), 9 of the 14 samples also showed LOH in the region, including the $p18^{INK4c}$ gene locus. Therefore, we looked for mutations of the $p18^{INK4c}$ gene by PCR-SSCP analysis; however, no mobility shifts were detected in the 30 cases (data not shown). The $p18^{INK4c}$ gene may not be affected in the transformation of CML. However, we cannot rule out the possibility that homozygous deletions of the gene had occurred, because we were unable to analyze these samples for the gene by Southern blot analysis.

Recently the *p73* gene was identified, and it maps to 1p36.[25] The gene is one of the candidates for tumor suppressor gene on 1p in neuroblastoma. However, the *p73* locus is more distal to the *D1S508* locus, and no p73 mutations were detected in neuroblastoma.[25] In addition, although we screened p73 mutations in various kinds of cell lines and fresh samples, no mutations were observed (unpublished data). Analysis of the expression will be helpful to understand the role of the *p73* gene in CML.

In 23 of the 30 samples, DNA was extracted from bone marrow smears and it probably contained both blast and chronic phase cells. Although LOH was found in a sample of a bone marrow aspirate containing 21% blast cells (CML no. 8), we may have underestimated the true incidence of LOH. Similarly, we may have missed homozygous deletions because of the possible contamination of chronic-phase cells in our BC or AP samples. Homozygous deletions for a polymorphic marker may appear as retention of heterozygosity as a consequence of amplification of DNA from the contaminating chronic phase cells.

Ph has been observed in more than 90% of CML cases. However, chromosomal deletions or monosomy of chromosome 1 has not been characterized in CML.[4,8,26,27] In our study, cytogenetic data were available from 29 patients. Because cytogenetic analysis did not show either 1p deletions or monosomy of chromosome 1 in any of the cases with LOH on 1p, our findings suggest that either mitotic nondisjunction with duplication or mitotic recombination is the frequent mechanism that resulted in LOH on 1p. The observation that heterozygosity was retained on 1q in 11 of 12 informative samples with LOH on 1p suggests that mitotic recombination is the most frequent mechanism of alteration. Small cytogenetically undetectable deletions may be present in some of the samples showing LOH on 1p. Nevertheless except for CML nos. 1, 11, and 24, the LOH covered such a large region that a deletion would have been detectable cytogenetically.

Cloning and characterization of the affected gene from chromosome arm 1p in progressive CML will help clarify the genesis of blastic transformation and provide insights into novel therapies.

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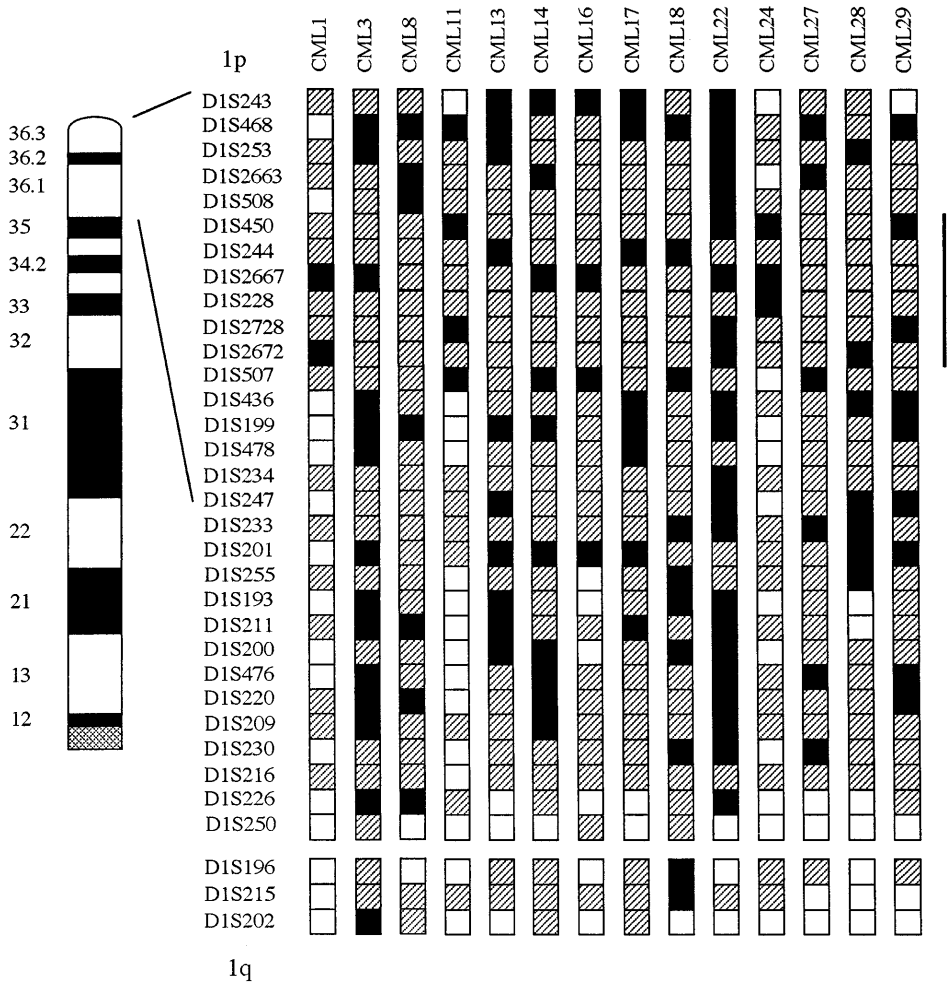

Fig 2. Deletional map of chromosome 1 in CML. The common region of LOH was located proximal to *D1S508* and distal to *D1S507*. Vertical bar indicates the common region of LOH. The locus symbols are shown on the left. Three loci are located on 1q (*D1S196*, *D1S215*, and *D1S202*). The sample name is on the top. Black box indicates LOH. White box indicates retained heterozygosity. Hatched box denotes not informative.

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REFERENCES

1. Weinberg RA: Tumor suppressor genes. Science 254:1138, 1991

2. Knudson AG: Mutation and cancer: Statistical study of retinoblastoma. Proc Natl Acad Sci USA 68:820, 1971

3. Kantarjian HM, Deisseroth A, Kurzrock R, Estrov Z, Talpaz M: Chronic myelogenous leukemia: A concise update. Blood 82:691, 1993

4. Kurzrock R, Gutterman JU, Talpaz M: The molecular genetics of Philadelphia-chromosome positive leukemias. N Engl J Med 319:990, 1988

5. Ahuja H, Bar-Eli M, Arlin Z, Advani SH, Allen SL, Goldman J, Snyder D, Foti A, Cline MJ: The spectrum of molecular alterations in the evolution of chronic myelocytic leukemia. J Clin Invest 87:2042, 1991

6. Sill H, Goldman JM, Cross NCP: Homozygous deletions of the p16 tumor-suppressor gene are associated with lymphoid transformation of chronic myeloid leukemia. Blood 85:2013, 1995

7. Towatari M, Adachi K, Kato H, Saito H: Absence of the human retinoblastoma gene product in the megakaryoblastic crisis of chronic myelogenous leukemia. Blood 78:2178, 1991

8. Melo JV: The molecular biology of chronic myeloid leukaemia. Leukemia 10:751, 1996

9. Vogelstein B, Fearon ER, Kern S, Hamilton SR, Preisinger AC, Nakamura Y, White R: Allelotype of colorectal carcinomas. Science 244:207, 1989

10. Tsuchiya E, Nakamura Y, Weng S-Y, Nakagawa K, Tsuchiya S, Sugano H, Kitagawa T: Allelotype of non-small cell lung carcinoma-comparison between loss of heterozygosity in squamous cell carcinoma and adenocarcinoma. Cancer Res 52:2478, 1992

11. Takeuchi S, Bartram CR, Wada M, Reiter A, Hatta Y, Seriu T, Lee E, Miller CW, Miyoshi I, Koeffler HP: Allelotype analysis of childhood acute lymphoblastic leukemia. Cancer Res 55:5377, 1995

12. Mori N, Morosetti R, Lee S, Spira S, Ben-Yehuda D, Schiller G, Landolfi R, Mizoguchi H, Koeffler HP: Allelotype analysis in the evolution of chronic myelocytic leukemia. Blood 90:2010, 1997

13. Sambrook J, Fritsch EF, Maniatis T: Molecular cloning: A Laboratory Manual. Plainview, NW, Cold Spring Harbor Lab Press, 1989

14. Litt M, Luty JA: A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:397, 1989

15. Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau P, Vaysseix G, Lathrop M: A second-generation linkage map of the human genome. Nature 359:794, 1992

16. White PS, Maris JM, Beltinger C, Sulman E, Marshall HN, Fujimori M, Kaufman BA, Biegel JA, Allen C, Hilliard C, Valentine MB, Look AT, Enomoto H, Sakiyama S, Brodeur GM: A region of consistent deletion in neuroblastoma maps within human chromosome 1p36.2-36.3. Proc Natl Acad Sci USA 92:5520, 1995

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17. Praml C, Finke LH, Herfarth C, Schlag P, Schwab M, Amler L: Deletion mapping defines different regions in 1p34.2-pter that may harbor genetic information related to human colorectal cancer. Oncogene 11:1357, 1995

18. Nagai H, Negrini M, Garter SL, Gillum DR, Rosenberg AL, Schwartz GF, Croce CM: Detection and cloning of a common region of loss of heterozygosity at chromosome 1p in breast cancer. Cancer Res 55:1752, 1995

19. Yeh S-H, Chen P-J, Chen H-L, Lai M-Y, Wang C-C, Chen D-S: Frequent genetic alterations at the distal region of chromosome 1p in human hepatocellular carcinomas. Cancer Res 54:4188, 1994

20. Cryns VL, Yi SM, Tahara H, Gaz RD, Arnold A: Frequent loss of chromosome arm 1p DNA in parathyroid adenomas. Genes Chromosom Cancer 13:9, 1995

21. Dracopoli NC, Harnett P, Bale SJ, Stanger BZ, Tucker MA, Housman DE, Kefford RF: Loss of alleles from the diatal short arm of chromosome 1 occurs late in melanoma tumor progression. Proc Natl Acad Sci USA 86:4614, 1989

22. Caron H, van Sluis P, de Kraker J, Bokkerink J, Egeler M, Laureys G, Slater R, Westerveld A, Voute PA, Versteeg R: Allelic loss of chromosome 1p as a predictor of unfavorable outcome in patients with neuroblastoma. N Engl J Med 334:225, 1996

23. Schwab M, Praml C, Amler LC: Genomic instability in 1p and human malignancies. Genes Chromosom Cancer 16:211, 1996

24. Guan K-L, Jenkins CW, Li Y, Nichols MA, Wu X, O'Keefe CL, Matera AG, Xiong Y: Growth suppression by p18, a $p16^{INK4/MTS1}$-and $p14^{INK4B/MTS2}$-related CDK6 inhibitor, correlates with wild-type pRb function. Genes Dev 8:2939, 1994

25. Kaghad M, Bonnet H, Yang A, Creancier L, Biscan J-C, Valent A, Minty A, Chalon P, Lelias J-M, Dumont X, Ferrara P, McKeon F, Caput D: Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. Cell 90:809, 1997

26. Rowley JD: Nonrandom chromosomal abnormalities in hematologic disorders of man. Proc Natl Acad Sci USA 72:152, 1975

27. Bernstein R: Cytogenetics of chronic myelogenous leukemia. Semin Hematol 25:20, 1988

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