Integration of human T-cell leukemia virus type 1 in genes of leukemia cells of patients with adult T-cell leukemia

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Adult T-cell leukemia (ATL) occurs after a long latent period of persistent infection by human T-cell leukemia virus type 1 (HTLV-1). However, the mechanism of oncogenesis by HTLV-1 remains to be clarified. It was reported that the incidence curve of ATL versus age was consistent with a multistage carcinogenesis model. Although HTLV-1 is an oncogenic retrovirus, a mechanism of carcinogenesis in ATL by insertional mutagenesis as one step during multistage carcinogenesis has not been considered thus far, because the exact integration sites on the chromosome have not been analyzed. Here we determined the precise HTLV-1 integration sites on the human chromosome, by taking advantage of the recently available human genome database. We isolated 25 integration sites of HTLV-1 from 23 cases of ATL. Interestingly, 13 (52%) of the integration sites were within genes, a rate significantly higher than that expected in the case of random integration (P=0.043, χ^2 test). These results suggest that preferential integration into genes at the first infection is a characteristic of HTLV-1. However considering that some of the genes are related to the regulation of cell growth, the integration of HTLV-1 into or near growth-related genes might contribute to the clonal selection of HTLV-1-infected cells during multistage carcinogenesis of ATL. (Cancer Sci 2004; 95: 306–310)

Adult T-cell leukemia (ATL) is an aggressive leukemia of T-cells.[1)] Although infection by a retrovirus, human T-cell leukemia virus type 1 (HTLV-1), is known to be an etiological factor, the precise role of HTLV-1 infection in leukemogenesis is still not clear.[2, 3)] However, ATL is induced in only a few percent of the infected population after 50–60 years of infection by HTLV-1, and statistical analysis of the incidence curve suggested a mechanism of multistep carcinogenesis consisting of 5 independent events.[4)]

HTLV-1 contains a unique *trans* acting viral gene called *tax*, and the function of Tax has been extensively analyzed. However, Tax alone is not sufficient to explain the pathogenesis of ATL.[5)] One of the unique characteristics of retroviruses is that they integrate in chromosomal DNA of the host. The significance of insertional mutagenesis in tumor formation has been well documented for oncogenic animal retroviruses.[6)] Two recent cases of leukemia arising from gene therapy for 11 boys with X-linked severe combined immune deficiency suggest the possibility of insertional mutagenesis by retroviral vector insertion near the *LMO2* gene that has been linked to leukemia.[7, 8)]

Although HTLV-1 is an oncogenic retrovirus, the pathogenesis of ATL by insertional mutagenesis has not been studied thus far, since the chromosomal integration sites of HTLV-1 vary greatly among patients.[9)] Previous studies of several ATL patients showed the preferential integration of HTLV-1 in AT-rich regions of the genome, but did not identify the precise integration sites in the genome.[10, 11)] Since the human genome sequence has become available in detail recently,[12, 13)] it has become possible to precisely analyze the sites of integration of HTLV-1 in the genome of ATL cells that show monoclonal proliferation.

Here we cloned 25 integration sites from 23 patients with ATL and identified their exact positions in the genome using the recently clarified human genome sequence. Interestingly, we found that 13 (52%) of the 25 integration sites were within genes, some of which have been reported to be related to cell growth.

Materials and Methods

Cases. Peripheral blood mononuclear cells were isolated from 23 patients with ATL and 4 asymptomatic HTLV-1 carriers, and their DNA was extracted with phenol and chloroform. The study was approved by the Ethics Committee of the University of Tsukuba.

Inverse PCR (I-PCR). The strategy to amplify the flanking sequence to the HTLV-1 provirus is shown in Fig. 1. Briefly, 1 μg of DNA was digested with *Pst*I for 2 h at 37°C, self-circularized with 8 units of T4 DNA ligase in a 200-μl reaction mixture for 2 h at 15°C, precipitated with ethanol and dissolved in TE buffer. The circularized DNA was used as a template for I-PCR. The reaction mixture contained Ex *Taq* polymerase (TaKaRa, Otsu) and one of the two sets of primers: U1641 (5′-CCCCTTTCCCTTTCATTCACGACTGACTGC-3′) and L60 (5′-AACACGTAGACTGGGTATCCGAAAAGAAGA-3′); or U992 (5′-TTAACGAAAAAGAGGCAGATGACAATGACC-3′) and L64 (5′-TCCAAACACGTAGACTGGGTATCCGAAAAG-3′). The conditions for I-PCR were 95°C for 1 min, followed by 35 cycles of 95°C for 30 s and 68°C for 7 min in a GeneAmp PCR system 9600 or 2400 (Applied Biosystems).

Cloning and sequencing. The PCR products were cloned into pGEM-T easy vector (Promega) and their sequences were determined using an ABI PRISM 377 (Applied Biosystems).

Identification of the integration sites. The sequences flanking the 3′LTR were compared to the DNA database and their chromosomal locations were determined by BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). All the candidate sites were confirmed using site-specific primers, which were located outside of the I-PCR-amplified region and do not contain known repeat elements. REPEATMASKER (Arian Smit, http://ftp.genome.washington.edu/cgi-bin/RepeatMasker) was used for extraction of repeat elements. The BLAST option of "Search for short, nearly exact matches" was used for examination of the specificity of primers. The PCR reaction mixture contained genomic DNA and Ex *Taq* polymerase with the U1641 forward primer and each reverse primer (Table 1). The

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conditions for genomic PCR were the same except for the annealing temperatures: 95°C for 10 min, then 35 cycles of 95°C for 30 s, the annealing temperature corresponding to each reverse primer (Table 1) for 1 min and 72°C for 5 min, followed by a final stage of 72°C for 7 min.

Statistical analysis. Goodness of fit was assessed using the χ^2 test.

Results

Isolation and confirmation of HTLV-1 insertion sites. For isolation of the integration sites of HTLV-1, we amplified and cloned the sequences of 25 integration sites from 23 ATL patients. There were 2 patients (patients 3 and 26) who gave 2 clones (03a and 03b; 26a and 26b, Table 2). In contrast, we could not amplify the sequence by I-PCR from 4 asymptomatic carriers of HTLV-1. This is consistent with the idea of monoclonal proliferation in ATL of leukemic cells that contain one or two copies of HTLV-1 provirus, while in carriers there is polyclonal proliferation with no major cell clones with HTLV-1 integration.[3] Therefore our I-PCR could not amplify DNA fragments from such a small population of infected cells in carriers.

The insertion sites were determined as follows. Nucleotide sequences of 200–500 bp derived from both ends of the I-PCR products were examined by "BLAST the Human Genome." A BLAST hit was regarded as identical when it matched the query with over 99% identity throughout the full length of the query sequence. First, if the query matched a single region of the human genome, the region was regarded as the insertion site. Second, if the query matched two regions, mismatches and

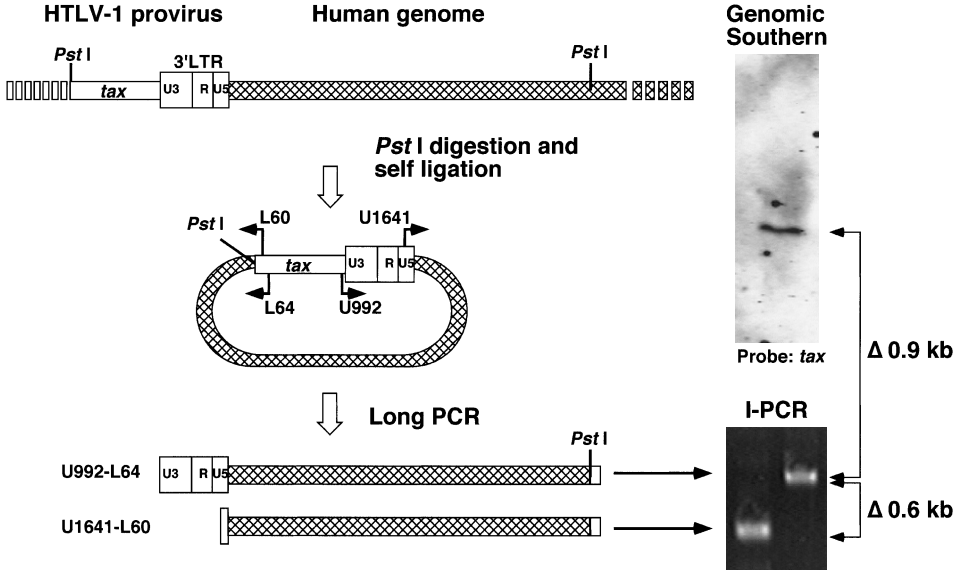

Fig. 1. Scheme of I-PCR to isolate the flanking sequences of HTLV-1 provirus. *Pst*I digest of genomic DNA was circularized with ligase, then subjected to PCR with 2 sets of primers as indicated. The specificity of I-PCR products was shown by the difference in size of 0.6 kb with the 2 sets of primers. The band on genomic Southern blot analysis showed a larger band by 0.9 kb than that of I-PCR product with U992-L64.

Table 1. Nucleotide sequences of the primers used to confirm the exact sites of integration

Clone ID	Reverse primers	Product size (bp)	Annealing temperature (°C)
05	5′-GAGAGAAAGGACAGTTGCCGATGCT	3285	64
10	5′-AGAGCCAAACAAAACCAAACATTAC	828	54
12	5′-ATCCCTATCTTGAAACCCCTTACCT	4902	68
13	5′-GCAGAACTAGGGCAGGCGGGGACAA	1641	60
15	5′-AGGATTGACCATAGGATTAGTGACC	2970	68
19	5′-AAACTATGATTGAACCTATTGAACG	3520	55
21	5′-TGTCCACAGTAAAATTCCTAGTGAG	3338	58
22	5′-TTCAACTTGTGACTGTACTTCCAGA	6129	68
24	5′-CTTGTCGGGGATTGAGATACCAGAG	1504	60
26b	5′-TCTAGTTCTACATCTTATCCAATCC	2079	58
27	5′-AGACAGTGGCAGTGGAGGCGAATAC	7136	64
34	5′-GGTAAGTAGTGGTTTTTTCAAGGT	1263	58
23	5′-GAGAGCCACCTACATTCCACAGACG	2093	68
04	5′-TGACTTCATCAAAGACTATTATTAA	4504	60
26a	5′-TGGACAAGGTGAGAAGTTTTAGGTA	2923	68
07	5′-GAACAAACCGAATCTGCTGCTCACC	1637	64
37	5′-TGGTGAAAAGAATAATATCTCCCGC	1339	68
41	5′-CTATCTCTACCCTTCAACCTATTCT	2578	68
01	5′-AAGACAGTTCCAGTTCCCAGATTGC	903	61
40	5′-TTCCATCTTCACCATAACAGTCCTT	2403	58
17	5′-GACCTAAGGAGAGACGGCAAGATGT	4925	66
03a	5′-TCTCAGGCAGCAGGCACAATACCAG	1166	64
03b	5′-TTCCCACAAAAACATCGCACCATCC	1091	61

Table 2. Summary of the HTLV-1 integration sites in clones isolated from patients with ATL

Clone ID	Classification[1]	and candidate genes with integration or nearest genes	Proviral direction[2]	LocusLink ID	Map	Local host sequences flanked with 3′-LTR of HTLV-1 provirus
05	I	CLIC5 (chloride intracellular channels 5) intron 1	+	53405	6p12.3	GCTGATAGCAGCAGAAACGG
10	I	androgen receptor intron 1	+	367	Xq11.2-12	GAAAGGCAGCCTGTTGCAAA
12	I	PRKG1 (protein kinase, cGMP-dependent, type I) intron 8	+	5592	10q11.2	CTTAGTCATTATAGGGCATA
13	I	myosin IF intron 1	+	4542	19p13.2	GTTAGGTGAGCTGCCCAATC
15	I	SPATA5 (spermatogenesis associated 5) intron 9	–	166378	4q27	CTCTTAGACCTAAGAAAAGA
19	I	HGNT-IV-H (UDP-*N*-acetylglucosamine: α-1,3-D-mannoside β-1,4-*N*-acetylglucosaminyltransferase IV-homolog) intron 4	–	25834	12q21	GCTTCCCATATATTTTCCTA
21	I	ZFD25 (zinc finger protein) intron 1	–	51427	7q11.2	AAATTGCATTGGGAACAAGG
22	I	HOOK3 (Golgi-associated microtubule binding protein) intron 15	+	84376	8p11.1	ACAGGGTTTTGCCCGGTTGG
24	I	DGKZ (diacylglycerol kinase, ζ) intron 6	+	8525	11p11.2	AAAAATGTGTTCAAAGCTGG
26b	I	EDG2 (endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2) intron 1	+	1902	9q32	GTTTGCGTGTGTATTTAGAA
34	I	SHARP (SMART/HDAC1 associated repressor protein) intron 5	+	23013	1p36.33-11	TTTATACGCCAAATTGAGAG
23	I	FBX15 (F-box only protein) intron 8	+	201456	18q22.3	GTACATGACTGTGTACTTCT
27	II	hypothetical Notch2-like transcript intron 2	+	NA[3]	1q21.2	GGAAGGGAGGCAGAAAATTT
01	III	16 kb up of FLJ20582 (hypothetical protein with multi Zn-finger)	+	54989	15q13.2	GGCTCATTCTTGTATTTTTA
03a	III	270 kb up of TMEPAI (transmembrane, prostate androgen induced RNA)	+	56937	20q13.31	CAGATCCTCTTCTGCTGAGC
03b	III	113 kb down of LOC139067 (similar to SPANX family, member C)	–	139067	Xq27.3	AACTTTTTCTTATTTAAATA
04	III	274 kb up of TGIF2LX	–	90316	Xq21.31	ACATATTACCATGGAAATGG
07	III	21 kb down of NRF1 (nuclear respiratory factor 1)	–	4899	7q32.2	CTGCGTGTTCCCCTTCCCTA
17	III	135 kb up of SEL1L	–	6400	14q31.1	GACATAAAAGTTCTCCAAGT
26a	III	47.6 kb up of SYN3	+	8224	22q12.3	AATACCCTCTTGGAGATGCC
37	III	59 kb up of TMEPAI	+	56937	20q13.31	GAAATGTCGTCCTAGCAACA
40	III	40 kb up of LOC253820 (similar to MEGF6, transcribed pseudogene)	+	253820	3q26.2	AGTATTAATTTGCTTTTGTT
41	III	78 kb up of GNAQ (guanine nucleotide binding protein (G protein), q polypeptide)	–	2776	9q21	TTAACTACTTATGAAGTTGA
02	IV	α-satellite	ND		ND	AGATTCCACAAAAAGAGTGT
08	IV	α-satellite	ND		ND	CAACTCTGTGAGTTGAATAC

1) The symbols represent: I, within known gene; II, within predicted gene; III, outside of gene. The nearest known or predicted gene is shown. IV: not specified.
2) Integration of the provirus at the 5′ or 3′ end of the gene, in sense (+) or antisense (–) orientation.
3) NA: LocusLink ID not available. Our assignment, predicted transcripts and peptides are different from annotations in data base. The exact insertion site of clone 27 corresponds to 452364-452369 of NT_004434.16, GI: 37547377.

gaps were compared carefully, and the candidate with an exact match was regarded as the insertion site. In fact, there are 2 candidates for clone 04, NCBI accession AL136111 on X and NCBI accession AC006152 on Y, and for clone 27, Notch2 on 1p13-p11 (NCBI accession AL512503) and Notch2-like transcript (NCBI accession AL592307) on 1q21.2. Third, if there was no hit, the query sequence was regarded as a repeat element. Re-analysis without the repeat element filter of "BLAST the Human Genome" and "BLASTn" showed many hits against repeat elements. However, we could determine the exact match by using unique junctions and nucleotide changes of the elements, except for 2 patients, in whom the sites of integration were located in the highly repetitive α-satellite sequences.

To further confirm the sites of integration in the leukemia cells from the original patients, we performed PCR using the forward primer within the 3′LTR, U1641, and the site-specific reverse primers deduced from the sequences of the respective integration sites isolated by I-PCR from 23 patients (Table 1). We observed bands with the expected sizes, as shown in Fig. 2.

Chromosomal location of the insertion sites. The flanking sequences of 25 integration sites are summarized in Table 2. The integration sites were found to be located on chromosomes 1p (2 sites), 3q, 4q, 6p, 7q (2 sites), 8p, 9q (2 sites), 10q, 11p, 12q, 14q, 15q, 18q, 19p, 20q (2 sites), 22q, and Xq (3 sites). Clones 02 and 08 were within highly repetitive α-satellite sequences on chromosomes 9 and 3, respectively. These results are consistent with previous reports indicating that there was no specific integration site in individual chromosomes,[9] although there were two cases with close integration at chromosome 20q13.31 (clones 03a and 37).

Preferential integration of HTLV-1 in genes. The genes in or around the insertion sites were identified according to the LocusLink description, except for predicted genes. We examined these predicted genes by comparison with EST records. A close examination of the integration sites on the human genome revealed that 52% (13/25) of integration sites were within transcriptional regions of genes (Table 2).

Discussion

Thirteen out of 25 integration sites (52%) of HTLV-1 in ATL patients were found in transcription units. According to the annotation of the human genome sequence, transcription units occupy about 33% of the genome.[12, 13] Therefore if HTLV-1 integrates randomly and there is no effect of integration into genes on cell proliferation, we should expect that 33% of inte-

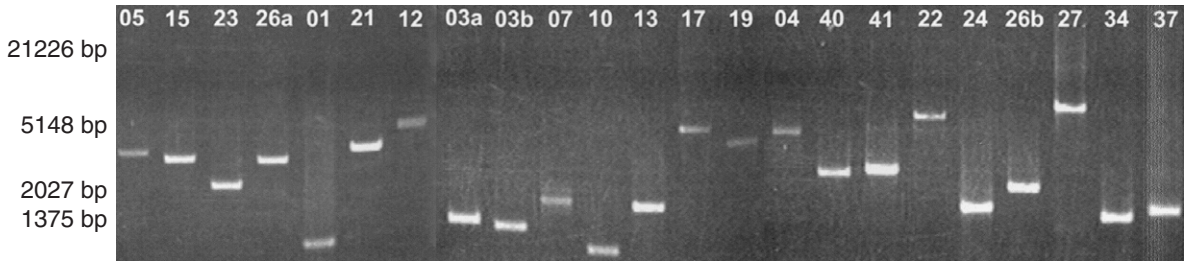

Fig. 2. Confirmation of the integration sites by PCR. The 23 integration sites other than the α-satellite repeats were confirmed by genomic PCR using forward primer on 3′LTR (U1641) and the corresponding reverse primers designed from each flanking sequence found in I-PCR (Table 1).

gration events would be within genes. However interestingly our results showed preferential integration of HTLV-1 into genes (P=0.043, χ^2 test). It is possible that HTLV-1 has the general property of preferentially initially integrating into transcription units and/or that the cell clones that have proviral integration within or near certain genes gain a growth advantage during carcinogenesis. Leclercq *et al*. have analyzed the host sequences flanked with HTLV-1 provirus among HTLV-1-infected individuals, but did not find preferential integration into genes.[11] At that time, information about the human genome sequence was not fully available, and the short range of the PCR method was not suitable for identification of the exact locus of integration in the human genome. We took advantage of the currently available human genome information and the long-range PCR method, and could identify the exact locations of HTLV-1 integration sites in ATL patients.

Interestingly, the sites of integration observed included several genes related to cancers (Table 2, classification I). They were the *SHARP* gene (clone 34) encoding a hormone-inducible SMART/HDAC1-associated repressor involved in the Notch/RBP-Jκ signaling pathway[14] and the Notch2-related gene (clone 27). It was reported that activation of the Notch signaling pathway may be related to leukemia.[15] In addition, there were the androgen receptor gene (clone 10) which regulates the expression of fibroblast growth factor 8,[16] the *diacylglycerol kinase ζ* gene (clone 24), a negative regulator of T cell activation,[17] and the *cGMP-dependent protein kinase* gene (clone 12), the expression of which correlates with cell density.[18] It was also reported that the insertion of the retrovirus could influence the transcription of adjacent genes, which were located within 300 kb of the integration site.[19] The distance between the integration sites of clones 03 and 37 was about 200 kb and the sites were close to the *PMEPA1* gene (also called the *TMEPAI* gene), a highly androgen-induced gene[20] and the target of TGFβ, which is involved in colon cancer.[21] The effect of proviral integration on gene expression was not fully characterized in the present work. However it is interesting that, in a cell line established from an ATL patient, the transcription and translation of IL-15 was enhanced by putative proximal integration of HTLV-1 provirus and consequent overproduction of IL-15.[22] Moreover, we examined transcription of a fused gene that had resulted from integration of HTLV-1 in or near the gene. In an ATL case with HTLV-1 integration 1 kb upstream from the *NY-ESO-1* gene, we detected a fused mRNA of the HTLV-1 LTR and exon 3 of the *NY-ESO-1* gene (Mizoguchi I, unpublished). These observations indicate that the integration of HTLV-1 can activate expression of the flanking genes.

It is conceivable that, during a long latent period of infection, cell clones with insertion of HTLV-1 into certain genes could acquire a further growth advantage when exposed to additional collaborating genetic and/or epigenetic changes. Li *et al*. have reported a powerful strategy to isolate many candidate leukemia disease genes through insertional mutagenesis with murine leukemia virus.[23] Although we do not know yet how many signal transduction pathways and involved genes are present that could explain the pathogenesis of ATL, accumulation of information about integration sites, along with information concerning other genetic and epigenetic changes, could afford a better understanding of the signal transduction pathways involved in multistage carcinogenesis.

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1. Uchiyama T, Yodoi Y, Sagawa K, Takatsuki K, Uchino H. Adult T-cell leukemia. Clinical and hematologic features of 16 cases. *Blood* 1997; **50**: 481–92.
2. Poiesz BJ, Ruscetti GW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci USA* 1980; **77**: 7415–9.
3. Yoshida M, Seiki M, Yamaguchi K, Takatsuki K. Monoclonal integration of human T-cell leukemia provirus in all primary tumors of adult T-cell leukemia suggests causative role of human T-cell leukemia virus in the disease. *Proc Natl Acad Sci USA* 1984; **81**: 2534–7.
4. Okamoto T, Ohno Y, Tsugane S, Watanabe S, Shimoyoma M, Tajima K, Miwa M, Shimotohno K. Multi-step carcinogenesis model for adult T-cell leukemia. *Jpn J Cancer Res* 1989; **80**: 191–5.
5. Yoshida M. Multiple viral strategies of HTLV-1 for dysregulation of cell growth control. *Annu Rev Immunol* 2001; **19**: 475–96.
6. Hayward WS, Neel BG, Astrin SM. Activation of a cellular onc gene by promoter insertion in ALV-induced lymphoid leucosis. *Nature* 1981; **290**: 475–80.
7. Kaiser J. Seeking the cause of induced leukemias in X-SCID trial. *Science* 2003; **299**: 495.
8. Check E. A tragic setback. *Nature* 2002; **420**: 116–8.
9. Seiki M, Eddy R, Shows T, Yoshida M. Nonspecific integration of HTLV provirus genome into adult T-cell leukemia cells. *Nature* 1984; **309**: 640–2.
10. Chou KS, Okayama A, Su IJ, Lee TH, Essex M. Preferred nucleotide sequence at the integration target site of human T-cell leukemia virus type I from patients with adult T-cell leukemia. *Int J Cancer* 1996; **65**: 20–4.
11. Leclercq I, Mortreux F, Cavrois M, Leroy A, Gessain A, Wain-Hobson S, Wattel E. Host sequences flanking the human T-cell leukemia virus type 1 provirus *in vivo*. *J Virol* 2000; **74**: 2305–12.
12. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, Fitzhugh W *et al*. Initial sequencing and analysis of the human genome. *Nature* 2001; **409**: 860–921.
13. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA *et al*. The sequence of the human genome. *Science* 2001; **291**: 1304–51.
14. Oswald F, Kostezka U, Astrahantseff K, Bourteeele S, Dillinger K, Zehner U, Ludwig L, Wilda M, Hameister H, Knochel W, Liptay S, Schmid RM. SHARP is a novel component of the Notch/RBP-Jκ signaling pathway. *EMBO J* 2002; **21**: 5417–26.

15. Aster JC, Pear WS. Notch signaling in leukemia. *Curr Opin Hematol* 2001; **8**: 237–44.
16. Gnanapragasam VJ, Robson CN, Neal DE, Leung HY. Regulation of FGF8 expression by the androgen receptor in human prostate cancer. *Oncogene* 2002; **21**: 5069–80.
17. Zhong XP, Hainey EA, Olenchock BA, Zhao H, Topham MK, Koretzky GA. Regulation of T cell receptor-induced activation of the ras-ERK pathway by diacylglycerol kinase ζ. *J Biol Chem* 2002; **277**: 31089–98.
18. Cornwell TL, Soff GA, Traynor AE, Lincoln TM. Regulation of the expression of cyclic GMP-dependent protein kinase by cell density in vascular smooth muscle cells. *J Vasc Res* 1994; **31**: 330–7.
19. Lazo PA, Lee JS, Tsichlis PN. Long-distance activation of the Myc protooncogene by provirus insertion in Mlv-1 or Mlvi-4 in rat T-cell lymphomas. *Proc Natl Acad Sci USA* 1990; **87**: 170–3.
20. Xu LL, Shanmugam N, Segawa T, Sesterhenn IA, McLeod DG, Moul JW, Srivastava S. A novel androgen-regulated gene, PMEPA1, located on chromosome 20q13 exhibits high level expression in prostate. *Genomics* 2000; **66**: 257–63.
21. Brunschwig EB, Wilson K, Mack D, Dawson D, Lawrence E, Willson JK, Lu S, Nosrati A, Rerko RM, Swinler S, Beard L, Lutterbaugh JD, Willis J, Platzer P, Markowitz S. PMEPA1, a transforming growth factor-beta-induced marker of terminal colonocyte differentiation whose expression is maintained in primary and metastatic colon cancer. *Cancer Res* 2003; **63**: 1568–75.
22. Bamford R N, Battiata AP, Burton JD, Sharma H, Waldmann TA. Interleukin (IL) 15/IL-T production by the adult T-cell leukemia cell line HuT-102 is associated with a human T-cell lymphotrophic virus type I R region/IL-15 fusion message that lacks many upstream AUGs that normally attenuate IL-15 mRNA translation. *Proc Natl Acad Sci USA* 1996; **93**: 2897–902.
23. Li J, Himmel KL, Dupuy AJ, Largaespada DA, Nakamura T, Shaughnessy JD Jr, Jenkins NA, Copeland NG. Leukemia disease genes: large-scale cloning and pathway predictions. *Nat Genet* 1999; **23**: 348–53.