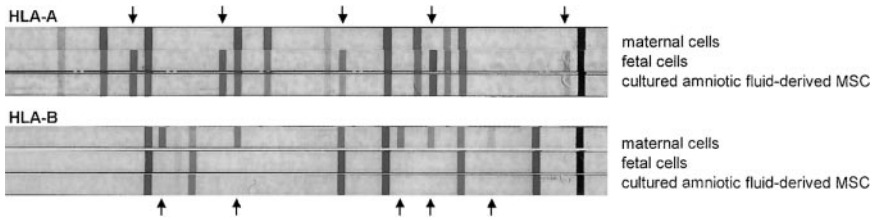

Figure 1. The Dynal Reli SSO (Dynal Biotech, Hamburg, Germany) reverse line blot strip assay was used for molecular typing of the HLA-A and HLA-B locus alleles of maternal cells, fetal cells, and culture-expanded amniotic fluid–derived MSCs from the same sample. The HLA-A and HLA-B type of the culture-expanded amniotic fluid–derived MSCs is identical to the fetal HLA-A and HLA-B type and mismatched with the maternal HLA-A and HLA-B type. Upward arrows indicate maternal specific HLA antigens, and downward arrows indicate fetal-specific HLA antigens.

Cotransplantation of UCB and haploidentical MSCs derived from parental BM is proposed as a strategy to reduce the delay in engraftment that is associated with UCB transplantation. The presence of MSCs in second-trimester amniotic fluid enables the possibility of cotransplantation of hematopoietic stem cells and MSCs from the same donor. This could be particularly useful in the setting of UCB transplantation between siblings.

Pieternella S. in 't Anker, Sicco A. Scherjon, Carin Kleijburg-van der Keur, Willy A. Noort, Frans H. J. Claas, Roelof Willemze, Willem E. Fibbe, and Humphrey H. H. Kanhai

Correspondence: W. E. Fibbe, Department of Hematology, C2R, PO Box 9600, 2333 AA Leiden, The Netherlands; e-mail: fibbe.hematology@lumc.nl

We thank W. Beekhuizen of the Center of Human Reproduction in Leiden for collecting the amniotic fluid.

References

1. Noort WA, Kruisselbrink AB, in 't Anker PS, et al. Mesenchymal stem cells promote engraftment of human umbilical cord blood-derived CD34(+) cells in NOD/SCID mice. Exp Hematol. 2002;30:870-878.
2. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284:143-147.
3. Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood. 2001;98:2396-2402.
4. Erlich H, Bugawan T, Begovich AB, et al. HLA-DR, DQ and DP typing using PCR amplification and immobilized probes. Eur J Immunogenet. 1991;18: 33-55.
5. Simmons PJ, Przepiorka D, Thomas ED, Torok-Storb B. Host origin of marrow stromal cells following allogeneic bone marrow transplantation. Nature. 1987; 328:429-432.
6. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol. 2000;109:235-242.
7. Mareschi K, Biasin E, Piacibello W, Aglietta M, Madon E, Fagioli F. Isolation of human mesenchymal stem cells: bone marrow versus umbilical cord blood. Haematologica. 2001;86:1099-1100.
8. Almeida-Porada G, Porada CD, Tran N, Zanjani ED. Cotransplantation of human stromal cell progenitors into preimmune fetal sheep results in early appearance of human donor cells in circulation and boosts cell levels in bone marrow at later time points after transplantation. Blood. 2000;95:3620-3627.
9. Frassoni F, Labopin M, Bacigalupo A. Expanded mesenchymal stem cells (MSC), co-infused with HLA identical hemopoietic stem cell transplants, reduce acute and chronic graft versus host disease: a matched pair analysis [abstract]. Bone Marrow Transplant. 2002;29:S2.

To the editor:

In B-cell chronic lymphocytic leukemias, 7q21 translocations lead to overexpression of the *CDK6* gene

Abnormalities of chromosome 7q have rarely been reported in chronic B-cell lymphoproliferative disorders.[1] Chromosomal translocation leading to overexpression of the *CDK6* gene, which is located in 7q21, has recently been described in splenic marginal zone lymphoma (SMZL).[2,3] CDK6 together with CDK4 are serine/threonine kinases that positively regulate the G1 phase progression in association with D-type cyclin. Overexpression of CDK6 is considered to contribute to leukemogenesis through dysregulation of cell proliferation, thereby favoring progression through the G1 phase. It seems likely that CDK6 overexpression is implicated in lymphomas with low proliferative index and, as we show here, may also be involved in other low-grade malignancies such as B-cell chronic lymphocytic leukemia (B-CLL). We have previously described the molecular characterization of a t(7;14)(q21;q32)

Table 1. Clinical and molecular characteristics of B-CLL patients with the CDK6 rearrangement

Patient	Sex/age at diagnosis, y	Overall survival (y of follow-up)	Karyotype	FISH analysis		SB analysis
				CDK6	Ig	
1	M/61	Alive (6)	46,XY,t(7;22)(q22;q11), del(13)(q13q14)[12]/46,XY[9]	Split	Igλ split	R
2	M/79	Alive (3)	46,X,−Y,+12[1]/46,X,−Y,+12, t(7;14)(q21;q32)[16]/46,XY[3]	Split	IgH split	NA
3	M/61	Died (16)	45,XY,t(2;7)(p11-12;q22),der(8) t(8;17)(p12;p11), −17[5]/47,XY, t(2;7),+5,+12[10]/46,XY[35]	Split	NA	R

FISH analysis was sequentially performed on the same mitosis with mixed Human BAC Clone GS119p5 and Clone GSI-552A1 (encompassing intron 1, exon 1, and 178 kbp upstream of the coding sequence of the *CDK6* gene; Incyte Genomics, San Francisco, CA) and with IgH probes (Vysis, Downers Grove, IL) or Igλ probes (kindly provided by Reiner Siebert). The *CDK6* gene was split between chromosomes 7 and 22 in FIO, between chromosomes 7 and 14 in DUP, and between chromosomes 2 and 7 in LAN. Igλ and IgH involvement was further demonstrated in patients 1 and 2, respectively. It may be noticed that the CDK6 GS119p5 probe gave a slight cross-hybridization with 3q chromosome. Southern blot analyses were performed with WS2 probe[4] and with *CDK6* PCR-amplified cDNA probes: E1 (nucleotides 12 to 298; GenBank accession no. X66365), p123, and p45 (nucleotides 446 to 860 and 61817 to 62434, respectively; GenBank accession no. AC002454). Restriction mapping allowed us to locate the breakpoint in patients 1 and 3 inside the breakpoint cluster region previously described within a 3.6 kbp upstream of the transcriptional start site of CDK6.[2] As no mitosis was available for FISH analysis in LAN, Igκ light chain locus involvement was confirmed by Southern blot analysis showing the cohybridization of the *CDK6* rearranged band with the Igκ probe (Jκ).

SB indicates Southern blot; R, rearranged; and NA, not available.

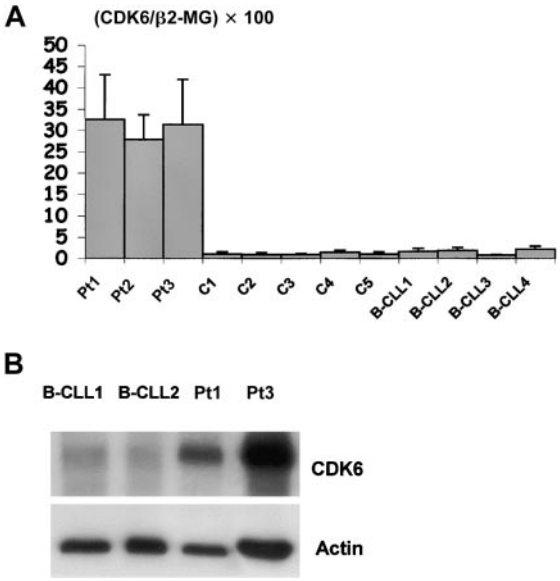

Figure 1. Overexpression of CDK6. (A) Quantitative real-time PCR was performed using the LightCycler system with FastStart DNA Master SYBR Green I kit according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). Primers from the *CDK6* gene are as follows: forward (5′-AGAAGAAGACTGGCCTAGAG) and reverse (5′-TGGAAGTATGGGTGAGACAGG). Primers from the β-2-microglobulin (*β2-MG*) gene used to evaluate correcting target molecule amounts are as follows: forward (5′-CCAGCAGAGAATGGAAAGTC) and reverse (5′-GATGCTGCTTACATGTCTCG). Amplification of specific transcripts was confirmed by a melting curve analysis. For PCR calibration, a serial 10-fold dilution series for each gene (ranging from 10^6 to 10 copies) was amplified and the assay was found to be linear over at least 5 orders of magnitude (*CDK6* slope, −3.47; *β2-MG* slope, −3.43). The absolute copy amount of each sample was obtained by the mean of the following ratio: ([copy number of the *CDK6* gene]/[copy number of *β2-MG*]) × 100. (B) Total cellular proteins were analyzed by Western blotting using anti-CDK6 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). The same blot was probed for actin to control for equal protein loading. In all samples, tumor cells represented more than 80% of the nucleated cells. RNA and protein were extracted from bone marrow or blood samples from patients with the *CDK6* gene rearrangement (FIO, DUP, LAN), from patients with B-CLL without the *CDK6* gene rearrangement (B-CLL1 to B-CLL4), and from normal mononuclear peripheral blood cells (C1 to C5).

observed in a case of B-CLL. The t(7;14) involved the immunoglobulin heavy chain (*IgH*) locus in 14q32 and a transposon-like element located 29 kbp upstream of the *CDK6* coding sequence.[4] However, no material was available for CDK6 expression analysis in this case. To analyze CDK6 involvement in B-CLL, we have examined the status of the *CDK6* gene in bone marrow or blood samples from a further 8 adult patients with B-CLL carrying cytogenetic abnormalities (deletions, duplications, or translocations) involving bands 7q21 to 7q22 (1.5% of our B-CLL series).

B-CLL diagnosis was established according to the World Health Organization Classification of Tumours and was characterized by positivity for CD5 and CD23 expression. All the tumor samples were screened, where possible, by Southern blot, fluorescence in situ hybridization (FISH), reverse-transcriptase real-time quantitative polymerase chain reaction (RT-RQ-PCR), and immunoblotting. In 3 patients, involvement of the *CDK6* gene was found by at least 2 different methods. The relevant clinical, biologic, and molecular data for these patients are given in Table 1. FISH and Southern blot analysis showed that an interesting common feature was that in all 3 cases the *CDK6* gene was rearranged by reciprocal translocations involving either *Igκ*, *Igλ*, or *IgH* locus and not with only the *Igκ* light chain locus as has previously been reported in other series.[2,3]

Marked overexpression of CDK6 was observed in all 3 patients with *CDK6* rearrangement compared with normal peripheral blood lymphocytes and with B-CLL without *CDK6* involvement (Figure 1). This indicates that FISH and CDK6 expression analysis can be successfully used to identify patients with *CDK6* involvement. As previously reported,[2] CDK6 overexpression can result from aberrant variable-diversity-joining (VDJ) or variable-joining (VJ) recombination, leading to the juxtaposition of the *CDK6* gene with the Ig gene enhancer during B-cell differentiation. In addition, our findings demonstrate the involvement of the *CDK6* gene in B-CLL, underlying that those abnormalities are not restricted to SMZL.

Sandrine Hayette, Isabelle Tigaud, Evelyne Callet-Bauchu, Martine Ffrench, Sophie Gazzo, Kamal Wahbi, Mary Callanan, Pascale Felman, Charles Dumontet, Jean-Pierre Magaud, and Ruth Rimokh

Correspondence: Sandrine Hayette, Laboratoire d'Hématologie et de cytogénétique, Hôpital Ed Herriot and INSERM U590, Lyon, France; e-mail: sandrine.hayette@chu-lyon.fr

References

1. Oscier DG, Gardiner A, Mould S. Structural abnormalities of chromosome 7q in chronic lymphoproliferative disorders. Cancer Genet Cytogenet. 1996;92: 24-27.
2. Corcoran MM, Mould SJ, Orchard JA, et al. Dysregulation of cyclin dependant kinase 6 expression in splenic marginal zone lymphoma through chromosome 7q translocations. Oncogene. 1999;18:6271-6277.
3. Brito-Babapulle V, Gruszka-Westwood AM, Platt G, et al. Translocation t(2; 7)(p12;q21–22) with dysregulation of the CDK6 gene mapping to 7q21–22 in a non-Hodgkin's lymphoma with leukemia. Haematologica. 2002;87:357-362.
4. Wahbi K, Hayette S, Callanan M, et al. Involvement of a human endogenous retroviral sequence (THE-7) in a t(7;14)(q21;q32) chromosomal translocation associated with a B cell leukemia. Leukemia. 1997;11:1214-1219.

To the editor:

Evaluating the relevance of the platelet transcriptome

A recent article in *Blood* by Gnatenko et al[1] globally profiled mRNA expression in platelets. Using microarray analysis, the authors reported that approximately 2000 transcripts (13%-17% of probed genes) are present in unstimulated platelets isolated from healthy human subjects and concluded that evaluating the platelet transcriptome will be useful for identifying proteins that regulate normal and pathologic platelet and megakaryocyte functions. We have also found similar transcript profiles in platelets with different microarray strategies.[2,3]

An accompanying commentary voiced similar conclusions, but also suggested that the most frequent platelet mRNAs detected by microarray are well-known leukocyte or red cell messages, heightening suspicion that their abundance may be due to contamination by these classes.[4] These 3 messages were thymosin β_4, neurogranin, and β-globin.[1] Thymosin β_4 and neurogranin protein are also found in platelets,[1,5] however, which is consistent with observations that platelets express gene products that are also present in other cell lineages.[2,3,6,7] Moreover, Gnatenko et al[1] took rigorous