Translocations t(X;14)(q28;q11) and t(Y;14)(q12;q11) in T-cell prolymphocytic leukemia

F. M. DE OLIVEIRA*, L. G. TONE†, B. P. SIMÕES*, E. M. REGO*, A. F. MARINATO*, R. H. JÁCOMO*, R. P. FALCÃO*

*Department of Internal Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil
†Department of Pediatrics, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil

Correspondence:
F. M. de Oliveira, Department of Internal Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, Av. Bandeirantes 3900, 14049-900 Ribeirão Preto, SP, Brazil. Tel.: +55 16 3602 2671; Fax: +55 16 3633 6695; E-mail: morato.oliveira@yahoo.com.br

doi:10.1111/j.1751-553X.2008.01036.x

Received 8 July 2007; accepted for publication 27 November 2007

Keywords
t(X;14)(q28;q11) and spectral karyotyping (SKY), t(Y;14)(q12;q11), T-prolymphocytic leukemia (T-PLL) and spectral karyotyping (SKY)

SUMMARY

We report a case of T-cell prolymphocytic leukemia (T-PLL) in a 41-year-old male. Classical cytogenetic, spectral karyotyping (SKY) and fluorescence *in situ* hybridization (FISH) studies of a blood sample obtained at diagnosis revealed the co-existence of t(X;14)(q28;q11), t(Y;14)(q12;q11) and a ring chromosome derivated from i(8)(q10). Immunophenotypic studies revealed involvement of T-cell lineage, with proliferation of $CD4^-$ $CD8^+$. The co-existence of two translocations involving both sex chromosomes in a case of T-PLL is rare. Chromosomal instability associated with the disease progression may have allowed the emergence of cell clones with translocations involving the sex chromosomes and the ring chromosome observed.

INTRODUCTION

T-cell prolymphocytic leukemia (T-PLL) is a malignant proliferation of lymphoid cells with a mature post-thymic phenotype. The disease is characterized by lymphadenopathy, splenomegaly, skin lesions, high lymphoid cell count and often refractoriness to conventional therapy (Jaffe *et al.*, 2001). Immunophenotyping studies showed that 65–70% of the cases are $CD4^+$ $CD8^-$, 15–20% co-express CD4 and CD8, 10% are $CD4^-$ $CD8^+$ and few are $CD4^-$ $CD8^-$. Morphologically, the tumor cells are medium-sized, atypical lymphocytes with irregular nuclei and prominent central nucleoli. In addition, a small-cell variant with less

atypical lymphoid cells may be recognized in 20% of patients (Jaffe *et al.*, 2001).

Classical cytogenetic studies have revealed the presence of complex karyotypes and some recurrent chromosomal abnormalities, the most frequent being t(14;14)(q11;q32), inv(14)(q11q32), t(X;14)(q28;q11), i(8)(q10) and t(8;8)(p12;q11) (Maljaei *et al.*, 1998). Other chromosomal abnormalities less frequently described are deletions or translocations of 6q, 12p, 13q, 17p and monosomy 22 (Soulier *et al.*, 2001). Nevertheless, analysis of cytogenetic abnormalities using spectral karyotyping (SKY), a technique based on the hybridization of 24 fluorescent labeled chromosome painting probes, has never been carried out in T-PLL. This technique is particularly useful for the analysis of complex and cryptic aberrations (Garini *et al.*, 1996).

We used classical cytogenetic, SKY analysis, and single color FISH to report the co-existence of t(Y;14)(q12;q11), t(X;14)(q28;q11) and a ring chromosome derived from i(8)(q10) in one case of T-PLL. To our knowledge, this is the first time that two specific translocations involving both sex chromosomes have been associated with a case of T-PLL.

CASE REPORT

A 41-year-old man was admitted to the University Hospital, School of Medicine of Ribeirão Preto, Brazil in May 2004. Clinical examination showed lymphadenopathy and hepatosplenomegaly. Peripheral blood counts showed: hemoglobin 12.5 g/dl, leucocytes 93.6×10^9/l, (75% of prolymphocytes) and platelets 128×10^9/l. DHL was 263 U/l. Immunophenotypic studies showed the following results: $CD1^-$, $CD2^+$, $CD3^+$, $CD4^-$, $CD5^+$, $CD7^+$, $CD8^+$, $CD10^-$, $CD16^-$, $CD19^-$, $CD23^-$, $CD45RA^-$, $CD56^-$, $CD79b^-$, TCR α/β^+, $FMC7^-$, TdT^-, κ^- and λ^-. Morphologically, the leukemia cells were medium-sized, atypical lymphocytes with irregular nuclei and prominent central nucleoli. The patient received two cycles of Chlorambucil (4 mg/day) and Fludarabine (25 mg/m^2) for 5 days each one, with no response. The patient was then treated with seven weekly doses of Campath-1H (30 mg). After 5 months of therapy, there was complete regression of lymphadenopathy and hepatosplenomegaly, and the peripheral blood count was normal.

Cytogenetic studies were performed on a nonstimulated culture of peripheral blood lymphocytes in RPMI 1640 medium with 20% fetal calf serum. Chromosome preparations were acquired according to standard procedures and the subsequent cytogenetic analysis and interpretation were made according to Shaffer and Tommerup ISCN (2005). The karyotype established was 46,t(X;14)(q28;q11)[20], t(Y;14)(q12;q11)[20], r(8)(::qter→q10::q10→ qter::)[20], +mar[20][cp20] (Figure 1a). Slides for SKY were prepared by using the same fixed chromosome preparations, stored at −20 °C, as employed for G-banding analysis. Chromosome labeling was performed with the SKY fluorescent labeling kit (Applied Spectral Imaging, Migdal HaEmek, Israel) according to the manufacturer's protocol. Chromosomes were counterstained with DAPI (4′-6-Diamidino-2-phenylindole). Image acquisition was performed with a SD200 Spectracube (Applied Spectral Imaging Inc.) mounted on an Olympus BX-40 microscope (Olympus America Inc., Center Valley, Pennsylvania, USA) using a custom designed optical filter (SKY-1; Chroma Technology, Brattleboro, VT, USA). Automatic identification of chromosomes was based on the measurement of the spectrum for each chromosome. A minimum of ten metaphases were analyzed using the SKYVIEW 2.1 software (ASI, Carlsbad, CA, USA). Spectral karyotyping confirmed the t(X;14)(q28;q11), t(Y;14)(q12;q11), r(8) and the origin of the marker chromosome, as being part of chromosome 5 (Figure 1b). FISH analysis was performed on the same cell preparations using commercial WCP14 (SpectrumOrange) (Vysis, Downers Grove, IL, USA), according to the manufacturer's instructions. The cells were counter-stained with DAPI and viewed on an Olympus BX40 microscope equipped with the FISHVIEW software, version 4.5.0.05 (ASI, Carlsbad, CA, USA). The WCP14 probe recognized homology of three chromosomes: the normal 14, t(X;14)(q28;q11) and t(Y;14)(q12;q11), as previously detected by SKY (data not shown). Cytogenetic analysis performed during remission showed a normal karyotype.

DISCUSSION

Classical cytogenetic allows the characterization of chromosomal abnormalities with considerable precision. However, the identification of complex karyotypes, marker chromosomes that comprise two or more chromosomes or cryptic translocations may become impossible by using G-banding analysis. New

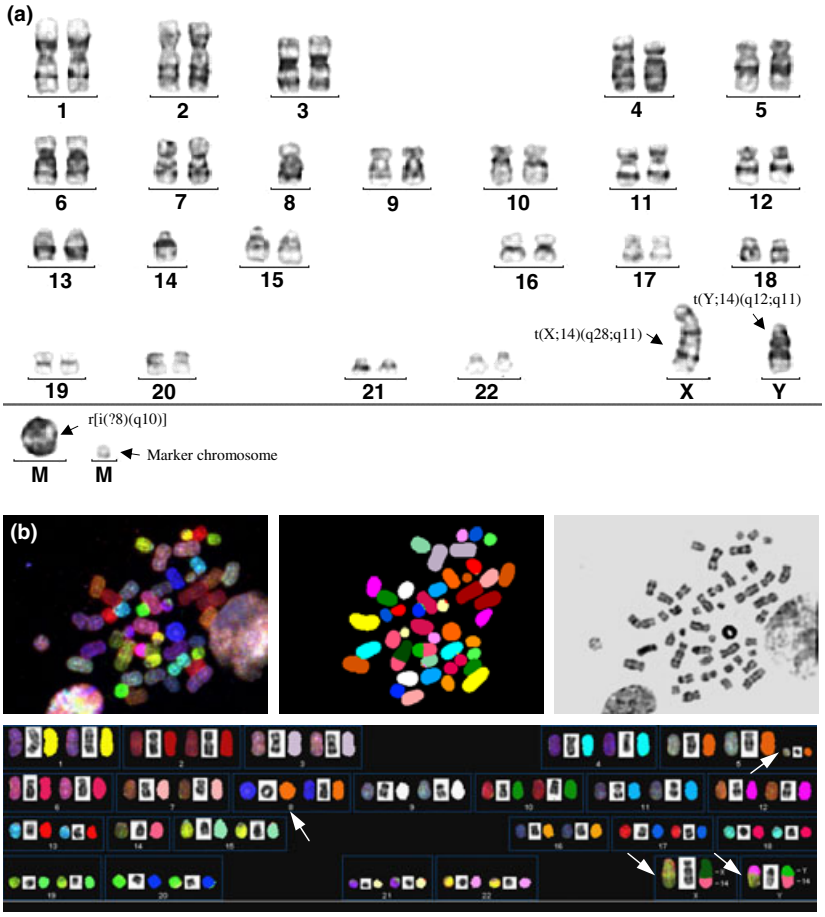

Figure 1. (a) Peripheral blood karyotype showing t(X;14)(q28;q11), t(Y;14)(q12;q11), r[i(?8)(q10)] and a marker chromosome (G-band with trypsin—Giemsa). (b) SKY analysis from a leukemic cell at time of diagnosis, showing the spectral image, classified image and inverted DAPI. SKY karyotype showing all of the profiles for each chromosome. Translocations involving the chromosomes X, Y and 14, the ring chromosome, r(8)(::qter→q10::q10→qter::), and the origin of the marker chromosome, as being part of chromosome 5.

techniques are therefore necessary to identify accurately the entire chromosome complement. SKY has been developed as a new generation fluorescence *in situ* hybridization (FISH) technique. By using SKY, it is possible to visualize each human chromosome in a different color using 24 chromosome painting probes. This appears to be a promising method for the analysis of unidentified chromosome abnormalities (Schröck *et al.*, 1996).

Classical and molecular cytogenetic analysis identified the co-existence of t(X;14)(q28;q11), t(Y;14)(q12;q11), a ring chromosome derived from i(8)(q10) and a marker chromosome derivated from chromosome 5 in a patient with diagnosis of T-PLL. The t(X;14)(q28;q11) and t(Y;14)(q12;q11) share the same breakpoint on chromosome 14 (14q11). According to Soulier *et al.* (2001), *TCR*α/δ genes located in 14q11 are involved in most of chromosomal abnormalities in cases of T-PLL. The t(X;14)(q28;q11) is associated with deregulation *MTCP-1 B1* gene (by juxtaposition to a T-cell receptor gene). Abnormalities involving the band Xq28 (the site for the *MCTP-1B1* gene, which has homology to *TCL-1* on 14q32.1) are common in T-PLL (Madani *et al.*, 1996). Rearrangements associated to *TCR* genes also result in juxtaposition of enhancer elements, responsible for *TCR* genes expression, next to different oncogene loci, leading to disruption of transcriptional pathways involved in normal T-cell development, and ultimately, leukemic transformation (Brito-Babapulle & Catovsky, 1991).

On the other hand, translocations involving the Y chromosome are rare events in hematological malignancies. The pseudoautosomal region of the Y chromosome, specifically Yq12 breakpoint, has been implicated in complex rearrangements involving Ph chromosome, in cases of CML (Dierlamm *et al.*, 1999).

The i(8)(q10) chromosome is a common finding in T-PLL (Mossafa *et al.*, 1994). However, the ring chromosome seen in this study has never been previously reported in T-PLL patients. The ring chromosome

structure originated as a consequence of a partial deletion of distal parts (telomeres) of both long arms of chromosome i(8)(q10), with subsequent rejoin. In general, ring chromosomes are unstable structures that rearrange when cells divide. Some studies pointed out to the presence of tumor suppressor genes on 8p (Kerangueven *et al.*, 1995; Berger & Busson, 2002). It has also been suggested that deletion or rearrangement of sequences localized in the short arm of chromosome 8 may be permissive for tumorigenesis in several cancers (Macoska *et al.*, 1995). The amplification of the q-arm of chromosome 8 accompanied by deletion of 8p sequences distal to the breakpoints could mean that two events may act synergistically to contribute to the malignant phenotype in T-PLL.

The investigation of chromosomal abnormalities and the localization of precise breakpoints involved in the complex rearrangements of T-PLL patients could improve our understanding of the genetic mechanisms underlying the progression of the disease.

REFERENCES

Berger R. & Busson M. (2002) Ring chromosomes 8 and translocation t(8;21) in a patient with acute myeloblastic leukemia. Annales de Génétique 45, 161–163.

Brito-Babapulle V. & Catovsky D. (1991) Inversions and tandem translocations involving chromosome 14q11 and 14q32 in T-prolymphocytic leukemia and T-cell leukemias in patients with ataxia telangiectasia. Cancer Genetics and Cytogenetics 55, 1–9.

Dierlamm J., Michaux L., Stefanova M., Eggert J., Leberecht P., Seeger D., Westerhausen M. & Hossfeld D.K. (1999) Novel Philadelphia variant t(Y;9;22)(q12;q34;q11) in a case of chronic myeloid leukemia. Cancer Genetics and Cytogenetics 15, 150–153.

Garini Y., Macville M., du Manoir S., Buckwald R.A., Lavi M., Katzir N., Wine D., Bar-Am I., Schrock E., Cabib D. & Ried T. (1996) Spectral karyotyping. Bioimaging 4, 65–72.

Jaffe E.S., Harris N.L., Stein H. & Vardiman J.W. (2001) WHO Classification of Tumours: Pathology and Genetics of Tumours of Haematopoietic and lymphoid tissues. IARC Press, Washington, DC.

Kerangueven F., Essioux L., Dib A., Noguchi T., Allione F., Geneix J., Longy M., Lidereau R., Eisinger F., Pebusque M.J., Jacquemier J., Boniti-Pellie C., Sobol H. & Brinbaum D. (1995) Loss of heterozygosity and linkage analysis in breast carcinoma: indication for a putative third susceptibility gene on the short arm of chromosome 8. Oncogene 10, 1023–1026.

Macoska J.A., Trybus T.M., Benson P.D., Sakr W.A., Grignon D.J., Wojno K.D., Pietruk T. & Powell I.J. (1995) Evidence for three tumor suppressor gene loci on chromosome 8p in human prostate cancer. Cancer Research 55, 5390–5395.

Madani A., Choukroun V., Soulier J., Cacheux V., Claisse J.F., Valensi F., Daliphard S., Cazin B., Levy V., Leblond V., Daniel M.T., Sigaux F. & Stern M.H. (1996) Expression of p13 MTCP-1 is restricted to mature T-cell proliferations with t(X;14) translocations. Blood 87, 1923–1927.

Maljaei S.H., Brito-Babapulle V., Hiorns L.R. & Catovsky D. (1998) Abnormalities of chomosomes 8, 11, 14, and X in T-prolymphocytic leukemia studies by fluorescence in situ hybridization. Cancer Genetics and Cytogenetics 103, 110–116.

Mossafa H., Brizard A., Huret J.-L., Brizard F., Lessard M., Guilhot F. & Tanzer J. (1994) Trisomy 8q due to i(8q) or der(8)t(8;8) is a frequent lesion in T-prolymphocytic leukaemia: four new cases and review of the literarure. British Journal of Haematology 86, 780–785.

Schröck E., du Manoir S., Veldman T., Schoell B., Wienberg J., Ferguson-Smith M.A., Ning Y., Ledbetter D.H., Bar-Am I., Soenksen D., Garini Y. & Ried T. (1996) Multicolor spectral karyotyping of human chromosomes. Science 273, 494–497.

Shaffer L.G., Tommerup N. & ISCN (2005) An International System for Human Cytogenetic Nomenclature. S. Karger Basel.

Soulier J., Pierron G., Vecchione D., Garand R., Brizard F., Sigaux F., Stern M.-H. & Aurias A. (2001) A complex pattern of recurrent chromosomal losses and gains in T-cell prolymphocytic leukemia. Genes, Chromosomes and Cancer 31, 248–254.