Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Francesco Lo Coco, Deputy Editor. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received June 30, 2003; accepted July 15, 2003.

References

1. van Dongen JJ, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia 1999; 13:1901-28.
2. Ottmann OG, Druker BJ, Sawyers CL, Goldman JM, Reiffers J, Silver RT, et al. A phase 2 study of imatinib in patients with relapsed or refractory Philadelphia chromosome-positive acute lymphoid leukemias. Blood 2002;100:1965-71.
3. Scheuring UJ, Pfeifer H, Wassmann B, Bruck P, Atta J, Petershofen EK, et al. Early minimal residual disease (MRD) analysis during treatment of Philadelphia chromosome/Bcr-Abl-positive acute lymphoblastic leukemia with the Abl-tyrosine kinase inhibitor imatinib (STI571). Blood 2003;101:85-90.
4. Fisher RA, Yates F. Statistical tables for biological, agricultural and medical research. Edinburgh, United Kingdom: Oliver and Boyd Ltd.; 1963. p. 46, Table 3.
5. Mitterbauer G, Nemeth P, Wacha S, Cross NC, Schwarzinger I, Jaeger U, et al. Quantification of minimal residual disease in patients with BCR-ABL-positive acute lymphoblastic leukaemia using quantitative competitive polymerase chain reaction. Br J Haematol 1999;106:634-43.
6. Brisco MJ, Sykes PJ, Hughes E, Dolman G, Neoh SH, Peng LM, et al. Monitoring minimal residual disease in peripheral blood in B-lineage acute lymphoblastic leukaemia. Br J Haematol 1997;99:314-9.
7. Martin H, Atta J, Bruecher J, Elsner S, Schardt C, Stadler M, et al. In patients with BCR-ABL-positive ALL in CR peripheral blood contains less residual disease than bone marrow: implications for autologous BMT. Ann Hematol 1994; 68:85-7.
8. van der Velden VH, Jacobs DC, Wijkhuijs AJ, Comans-Bitter WM, Willemse MJ, Hahlen K, et al. Minimal residual disease levels in bone marrow and peripheral blood are comparable in children with T cell acute lymphoblastic leukemia (ALL), but not in precursor-B-ALL. Leukemia 2002;16:1432-6.

A novel translocation t(14;15)(q32;q24) bearing deletion on der(14) in Philadelphia-positive chronic myeloid leukemia

We report the molecular cytogenetic characterization of a case of Philadelphia positive chronic myeloid leukemia (CML) with a t(14;15)(q32;q24) at onset, showing deletions on der(14) in addition to the loss of chromosome 22 sequences on der(9). To our knowledge, the presence of deletions on chromosomes involved in distinct and concomitant rearrangements other than t(9;22) has never been observed in CML patients.

haematologica 2003; 88:1076-1077
(*http://www.haematologica.org/2003_09/1076.htm*)

The Philadelphia chromosome (Ph) is the cytogenetic hallmark of chronic myeloid leukemia (CML) and is present in approximately 90–95% of patients.[1] The loss of genomic sequences on der(9) chromosome has been described in approximately 9% of CML patients. These deletions identify a subgroup with a worse prognosis.[2] Albano *et al.*[3] recently reported the occurrence of deletions on the third chromosome involved in variant complex t(9;22) translocations. In the present paper, we report the molecular cytogenetic characterization of a Ph+ CML case with a t(14;15)(q32;q24) at onset, showing deletions on der(14) in addition to the loss of chromosome 22 sequences on der(9). Conventional cytogenetic analysis of a 24-48 hour culture was performed on bone marrow cells at diagnosis by giemsa-trypsin-giemsa (GTG) banding. The patient was tested by reverse transcription polymerase chain reaction (RT-PCR) and fluorescence *in situ* hybridization (FISH).

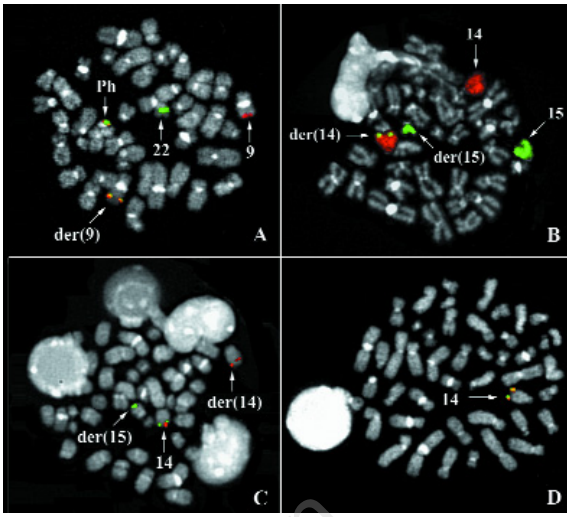

Figure 1. A) FISH co-hybridization with clones specific for ABL (red) and BCR (green) genes showed a fusion signal on both Ph and der(9) chromosomes. A faint BCR signal was observed on the der(9) chromosome, suggesting a partial deletion. B) WCP#14 (red) and WCP#15 (green) revealed the t(14;15) translocation. C) FISH experiments with clones RP11-796G6 (red) and RP11-356L8 (green) allowed us to map the breakpoint in 14q32.31. D) Clones RP11-350L3 (red) and RP11-114H15 (green), both mapping in 14q32.31, gave hybridization signals only on the normal 14 chromosome, indicating deletion of the chromosomal region encompassed by the two clones.

BAC RPCI-11 164N13 and a mixture of PACs RPCI-5 835J22 and 1132H12 were used to identify BCR and ABL genes, respectively.[3] Whole-chromosome paints (WCPs) for chromosomes 14 and 15, derived from flow-sorted chromosomes, were DOP-amplified. A series of 15 PAC/BAC clones specific for chromosomes 14 and 15 was used to define the t(14;15) breakpoints. Probe locations and gene mapping were derived from the University of California Santa Cruz database (November 2002 release). Primers specific for *WDR20* (exons 2: 5'TCTGCTGTGGCTGTTAGGTG3'; exon 3: 5'TTCCTCGTGGATTTGCTCTT3') and *PML*[4] (PML-C2: 5'AGCGCGACTACGAGGAGAT3') genes were selected and employed in the RT-PCR experiments.

In March 2002 a 64-year old female was admitted to our Institution with leukocytosis and splenomegaly. The diagnosis of chronic phase-CML was made. After initial cytoreduction obtained with hydroxyurea, the patient started Glivec therapy. She is now doing well in hematologic remission.

The t(14;15) translocation was detected in all analyzed metaphases, in addition to the classical t(9;22) rearrangement, providing the following karyotype: 46, XX, t(9;22)(q34;q11), t(14;15)(q32;q24)[25]. FISH experiments with probes specific

for ABL and BCR genes revealed a fusion signal on both der(22) and der(9) chromosomes. A fainter than expected signal of the BCR probe was observed on der(9), indicating the occurrence of a deletion. The use of appropriate BAC clones distal to BCR allowed us to identify a 1 Mb deletion of chromosome 22 sequences on der(9). Among 17 genes mapping within this deleted region there are 2 tumor suppressor genes (TSGs): *SMARCB1* (swi/snf-related, matrix-associated, actin-dependent regulator of chromatin, subfamily b, member 1) and *GSTT1* (glutathione S-transferase τ 1).[5-6]

FISH experiments using WCPs for #14 and #15 confirmed the involvement of these chromosomes in the t(14;15). The breakpoint on chromosome 14 was mapped between RP11-796G6 and RP11-356L8 (300 kb apart) giving a hybridization signal on the der(14) and der(15) chromosomes, respectively. Clones RP11-350L3 and RP11-114H15, localized between RP11-796G6 and RP11-356L8 probes, failed to reveal any signal on the derivative chromosomes, indicating deletion of the chromosomal region encompassed by the two clones.

Three genes with known function are located in the chromosome 14 deleted region: *DNCH1* (dynein, cytoplasmic, heavy polypeptide 1), *HSPCA* (heat shock 90kDa protein 1, HSP90, α) and *PPP2R5C* (protein phosphatase 2, regulatory subunit B (B56), γ isoform). *DNCH1* encodes a protein which has a role in mitotic spindle formation and may function as a motor protein.[7] *HSPCA* encodes the α subunit of HSP90 heat-shock proteins family, synthesized at increased rates in response to heat and other forms of stress.[8] The *PPP2R5C* gene encodes a regulatory B subunit of the protein phosphatase 2A (PP2A), also designated as B56; the PP2A enzyme is implicated in a variety of regulatory processes including cell growth and division, muscle contraction, and gene transcription. A recent study suggested that the γ isoform of B56 subunits can suppress tumor cell growth.[9]

The same experimental approach for chromosome 15 led to the identification of the breakpoint on clone RP11-247C2, specific for the *PML* gene (15q24), which gave a clear splitting signal on der(15) and der(14). As *WDR20* (WD repeat domain 20) gene10 maps in the clone RP11-356L8, transferred on der(15), the presence of a possible *5'PML/3'WDR20* fusion gene was evaluated. No amplification product was obtained excluding the presence of a new fusion gene involving *PML*. In our Ph+ CML case we found an additional, novel translocation t(14;15)(q32;q24) characterized by the loss of genomic sequences on der(14). The occurrence of deletions on chromosomes not involved in the t(9;22) rearrangement has never been reported in CML.

In conclusion, the detection of genomic sequence loss on der(14)t(14;15) suggested that reciprocal chromosomal translocations could be really unbalanced. Because of the poor knowledge of the function of some deleted genes, the significance of such deletions concomitant to those on der(9) is unclear.

Francesco Albano, Giorgina Specchia,*° Luisa Anelli,*# Antonella Zagaria,*# Arcangelo Liso,* Vincenzo Liso,* Mariano Rocchi#*

**Department of Hematology, University of Bari, Bari, Italy; °Hematology, University of Foggia, Foggia, Italy; #Department of Pathologic Anathomy and Genetics, Section of Genetics, University of Bari, Bari, Italy*

Key words: t(14;15); chronic myeloid leukemia; deletions.

Correspondence: Prof. Giorgina Specchia, Department of Hematology, University of Bari, Policlinico, Piazza G. Cesare 11, 70124 Bari, Italy. Phone: international +39.080.5478711. Fax: international +39.080.5428978. E-mail: emadhba@cimedoc.uniba.it

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received July 17, 2003; accepted August 3, 2003.

References

1. Mitelman F, Johansson B, Mertens F. (eds) 2002 Mitelman Database of Chromosome Aberrations in Cancer, WWW document. http://cgap.nci.nih.gov/Chromosomes/Mitelman.
2. Kolomietz E, Al-Maghrabi J, Brennan S, Karaskova J, Minkin S, Lipton J, et al. Primary chromosomal rearrangements of leukemia are frequently accompanied by extensive submicroscopic deletions and may lead to altered prognosis. Blood 2001;97:3581–8.
3. Albano F, Specchia G, Anelli L, Zagaria A, Storlazzi CT, Buquicchio C, et al. Genomic deletions on other chromosomes involved in variant t(9;22) chronic myeloid leukemia cases. Genes Chromosomes Cancer 2003;36: 353-60.
4. van Dongen JJ, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia 1999;13:1901-28.
5. Sevenet N, Lellouch-Tubiana A, Schofield D, Hoang-Xuan K, Gessler M, Birnbaum D, et al. Spectrum of hSNF5/INI1 somatic mutations in human cancer and genotype-phenotype correlations. Hum Mol Genet 1999;8:2359-68.
6. Haase D, Binder C, Bunger J, Fonatsch C, Streubel B, Schnittger S, et al. Increased risk for therapy-associated hematologic malignancies in patients with carcinoma of the breast and combined homozygous gene deletions of glutathione transferases M1 and T1. Leuk Res 2002;26: 249-54.
7. Narayan D, Desai T, Banks A, Patanjali SR, Ravikumar TS, Ward DC. Localization of the human cytoplasmic dynein heavy chain (DNECL) to 14qter by fluorescence in situ hybridization. Genomics 1994;22:660-1.
8. Ozawa K, Murakami Y, Eki T, Soeda E, Yokoyama K. Mapping of the gene family for human heat-shock protein 90-α to chromosomes 1, 4, 11, and 14. Genomics 1992; 12:214-20.
9. Muneer S, Ramalingam V, Wyatt R, Schultz RA, Minna JD, Kamibayashi C. Genomic organization and mapping of the gene encoding the PP2A B56g regulatory subunit. Genomics 2002;79:344-8.
10. Strausberg RL, Feingold EA, Grouse LH, Derge JG, Klausner RD, Collins FS, et al. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. Proc Natl Acad Sci USA 2002;99:16899-903.