Short communication

Molecular diagnostics of promyelocytic leukaemia

Janusz KOCKI¹, Maria CONSTANTINOU², Maria CIOCH³, Mirosław ŁAŃCUT⁴, Bogdan KALUŻEWSKI¹, Anna DMOSZYŃSKA³, Jacek WOJCIEROWSKI¹

¹Department of Medical Genetics, Medical University of Lublin, Lublin, Poland
²Department of Medical Genetics, Medical University of Łódź, Łódź, Poland
³Department of Haematology, Medical University of Lublin, Lublin, Poland
⁴Department of Histology and Embryology, Medical University of Lublin, Lublin, Poland

Abstract. Acute promyelocytic leukaemia (APL) is characterised by proliferation of abnormal promyelocytes. The reciprocal translocation between the long arms of chromosomes 15 and 17, and the fusion between the retinoic acid receptor (RARα) gene, and PML gene, is unique to APL. Because of unsuccessful cytogenetic analysis of conventional G-banding technique (mitoses were not observed), we diagnosed three non-treatment patients with APL by following molecular methods: reverse transcription–polymerase chain reaction (RT-PCR), fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH). At the time of diagnosis our patients showed reciprocal translocation t(15;17)(q22;q12) in all cases studied (66-85% of positive bone marrow cells). With the use of CGH we observed the unbalanced chromosomal aberrations: losses of 5q13.1, 5q31.3, 9p21 regions, gain of 5q32 region and trisomy of 18 chromosome.

Key words: RT-PCR, FISH, CGH, human acute promyelocytic leukaemia.

Acute promyelocytic leukaemia (APL) is an acute myeloid leukaemia with abnormal promyelocyte predomination. APL comprises 5-8% of all cases of acute myeloid leukaemias. The traditional approach to the diagnosis of APL includes clinical, cytochemical, immunophenotyping and cytogenetic analysis (LO COCO at al. 1999).

In this study, we used molecular methods for definite diagnosis of three patients with APL, in which the classic cytogenetic methods were unsuccessful (mitoses were absent).

Correspondence: J. KOCKI, Department of Medical Genetics, Medical University of Lublin, ul. Radziwiłłowska 11, 20-950 Lublin, Poland, e-mail: janusz.kocki@inetia.pl
The bone marrow cells were obtained from three (3) patients from the Department of Haematology of the Medical University of Lublin with APL suspicion. The clinical and immunological diagnoses were based on FAB classification – M3 type. The FISH technique was applied with an LSI PML(15q22)-SO/RARA(17q21)-SG probe (Vysis), according to the manufacturer’s instructions. Total cellular RNA was extracted from mononuclear bone marrow cells and RT–PCR was performed as described in an earlier study (KOCKI 2001). The primers were designed for each type of PML-RARα transcript: QP3 and QR1 for breakpoint cluster region 1 (Bcr1), while QP1 and QR1 for Bcr3 (O’CONNOR et al. 1999). The specificity of the PCR products was confirmed by detection with a PML-RARα probe (CASSINAT 2000). The CGH technique was employed according to the standard procedure (KALLIONIEMI et al. 1994).

Figure 1. Results of molecular and cytogenetic analyses of patients
Molecular methods (FISH, RT-PCR and CGH) were employed for genetic diagnosis of APL in three patients (before treatment). An analysis by FISH (Figure 1A) and RT-PCR (Figure 1B) showed reciprocal translocations t(15;17)(q22;q12) in all the cases at the time of diagnosis between 66-85% of positive bone marrow cells. All the patients were analysed by the CGH method. The following unbalanced chromosome aberrations were observed: loss of 5q13.1 (Figure 1C, patient 1), loss of 9p21 and 5q31.3 (Figure 1D, patient 2), and trisomy of 18 chromosome and loss of 5q32 (Figure 1E, patient 3). Our patients with APL were evaluated by FISH, RT-PCR, and CGH methods to determine genetic changes at the time of diagnosis (before treatment). The structural rearrangement, involving chromosomes 15 and 17, is unique to acute promyelocytic leukaemia. APL reveals a particular sensitivity to treatment with all-trans retinoic acid, which acts as a differentiating agent (JAFFE et al. 2001). The fusion protein, PML-RAR-α, causes a block in myeloid differentiation at the promyeloocyte stage, possibly by complexing and inactivating wild-type PML (ROBERTSON et al. 1992). CGH analysis of DNA from APL patients provides a potential for detecting occult genetic changes. CGH was widely used during the past 8-9 years to study chromosome aberrations and imbalances in solid tumours and haematological malignancies. CGH appears to be an important tool for the analysis of unbalanced chromosomal changes, particularly in those cases in which classic banding analysis of tumour karyotypes faces several problems, including a frequently poor growth rate of the leukaemic cells, resulting in a low yield of mitoses or inferior banding quality of the chromosomes. APL patients with chromosomal translocation t(15;17) only, are regarded as a favourable group among acute leukemias, mainly because of its unique sensitivity to all-trans retinoic acid (ATRA), yielding complete remission rates of 80-90% (HUANG et al. 1988). A subset of APL patients have, in addition to translocation t(15;17), some other chromosome aberrations, with incidence ranging from 29 to 43%: trisomy 8, iso(17q) and del(9q) (SLACK et al. 1997). The additional chromosome aberrations in APL cells (in our patients) may be of limited good prognostic value and its impact may depend largely on treatment protocols used (VARDIMAN et al. 2002).

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REFERENCES


