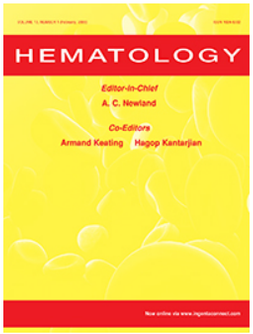

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Molecular Cytogenetic Analysis of B-CLL Patients with Aggressive Disease

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We tested a set of commercially available probes to determine the feasibility and accuracy of FISH in the detection of abnormalities in 13 patients with Chronic Lymphocytic Leukemia (CLL) with a particular aggressive clinical disease. We utilized three different probes for the 13q12–14 region, one for the centromeric region of chromosome 12, one for the P53 gene at 17p13.1 and one for 3′–5′ IGH at 14q32, covering the entire region of IGH, thus potentially allowing to detect more rearrangements. Conventional cytogenetic study showed a normal karyotype in 8/13 patients. FISH was able to detect chromosomal abnormalities in 10/13 pts (85%): +12 in 4 pts (38%); del 13q in 4 (38%); del 17p in 3 (35%); del of 5′-IGH in 1 (15%). In conclusion FISH confirmed its ability to improve the detection of cytogenetic abnormalities especially in patients with an aggressive disease.

Keywords: Cytogenetics; CLL; FISH; Prognosis group

INTRODUCTION

By standard cytogenetic analysis ~50% of the Chronic Lymphocytic Leukemia (CLL) cases studied are inadequate [1–5]. The application of FISH to CLL has recently enhanced our ability to detect chromosomal aberrations and reassessed the true incidence, providing new insights for a better understanding of the pathways of this disease. A large amount of data is available from the analysis of large series of CLL patients: trisomy 12 is observed as the first abnormality in ~20% of cases; deletions and, less frequently, translocations of 13q14 are present in ~40–50% of the cases; abnormalities of 11q22–23 and 6q21–23 in 20 and 5%, respectively; 14q32 alterations seem also to have a role in this disease, even if in a minority of cases, contrasting with initial reports [3,4]. Detection of genetic abnormalities used in the initial work-up of a CLL patient in many Hematology Divisions, allows a risk assessment at diagnosis and gives the opportunity for a risk adapted management. In fact trisomy 12 has been reported as a poor prognostic factor in the past, even if a more recent FISH study identified those patients carrying this abnormality as an intermediate prognosis group [3]. Deletion of chromosome 13q14 identifies a good prognosis group, while del of 17p has been reported as a poor prognostic feature, particularly associated with resistance to purine analog treatment. Here we report a molecular cytogenetic study of patients with an aggressive disease utilizing probes covering four different genomic regions. The aim of the study was twofold: to determine the accuracy and frequency of FISH in the detection of genetic abnormalities; to study a particular group of patients not usually described in the literature, i.e. those with a more active disease.

MATERIALS AND METHODS

Patient Population

We examined samples from 13 patients who were diagnosed and treated at the Hematology Division, University of Siena. The population comprised CLL patients with a normal karyotype by conventional cytogenetic analysis (CCy) with G-banding (8 patients), patients with an abnormal karyotype (4 patients), 1 patient did not have evaluable metaphases. We included all patients for whom

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stored material was available (cells processed for cytogenetic analysis).

Diagnosis and Disease Classification

The diagnosis and classification of CLL was based on morphological and immunophenotypic studies of peripheral blood smears,bone marrow aspirates and lymph node biopsy specimens obtained prior to therapy and at progression, according to standardized international criteria (NCI, IWCLL). Patients with an aggressive disease were defined by clinical progression characterized by lymph node bulky enlargement and/or lymphocyte doubling time inferior at 3 months, and/or resistance to therapy.

Cytogenetic and FISH Analysis

Cytogenetic analysis was performed with a G-banding technique on unstimulated 72 h cultures of peripheral blood cells in RPMI medium supplemented with 15% of fetal calf serum. Chromosomal abnormalities are described according to the International System for Cytogenetic Nomenclature (1995) [6]. We utilized different probes for the 13q14 region, in particular unique sequence specific probes for RB-1 (retinoblastoma), D13S319 locus and D13S25 (LSI-RB1, LSI D13S319, LSI D13S25, Vysis); one probe for chromosome 12 specific alpha-satellite DNA probe (CEP 12, Vysis); one probe for the P53 gene at 17p13.1 (LSI P53, Vysis); and one probe for the 3′–5′ IGH region at 14q32 (LSI IGH, Vysis). FISH was performed on fixed cells according to the manufacturer's specifications. Briefly, slides were denatured in 70% formamide/2 × SSC for 3 min at 75°C and dehydrated in ethanol solutions. About 10 μl of probes were hybridized on interphase cells overnight at 42°C and washes performed in 2 × SSC and Tween/2 × SSC, counterstained with 4′–6′-diamidine-2-phenylindole (DAPI). Slides were analyzed with a Nikon 2 fluorescence microscope and images captured with a CCD camera using image analysis system (Deltasistemi, Italy). At least 300 interphase cells were analyzed independently by two different observers. The cut-off for positivity was determined for each probe on five peripheral blood samples collected from normal individuals.

RESULTS AND DISCUSSION

A normal karyotype was observed by CCy in 8/13 patients studied. One additional patient did not show any analyzable metaphase at CCy. The abnormal karyotypes found in 4 patients are described in Table I; in particular one patient had a trisomy of chromosome 12, one patient a del(13q) and two patients had a complex karyotype. By FISH we found chromosomal abnormalities in 10/13 patients (85%): in particular a +12 was found in 4 patients (38%), range 47–58% of abnormal cells; del(13q) was found in 4 patients (38%), range 32–88% of abnormal cells; del(17p) in three patients (35%), range 34–68% of abnormal cells; del of 5′ IGH in one patient (15%), in 60% of the cells. Interestingly one patient showed both deletion of P53 at 17p and for all the probes covering the 13q region. One of three patients showing a del(13q) had a deletion of LSI D13S319 and D13S25 (so called DBM region) while RB-1 stayed at 13q14.

Interestingly 6/8 patients with a normal karyotype by CCy showed abnormalities by FISH.

In conclusion FISH is a powerful tool for the genetic analysis of CLL as it overcomes the low mitotic index of the CLL cells. Using FISH,

TABLE I CCy and FISH results

				−13			
Pt	Ccy	+12	−17P	(RB	D13S19	D13S25)	−14Q32
1	n	57%	–	–	–	–	–
2	+12	47%	–	–	32%	–	–
3	Complex	–	68%	–	–	–	–
4	13q−;17p−	–	34%	56%	62%	88%	–
5	n	58%	–	–	–	–	–
6	n	–	–	32%	–	–	–
7	Complex	–	–	–	35%	35%	–
8	n	–	61%	–	–	–	–
9	n	47%	–	–	–	–	–
10	n	–	–	–	–	–	60%
11	n	–	–	–	–	–	–
12	n	–	–	–	–	–	–
13	NA	–	–	–	–	–	–

Pt = patient number; CCy = conventional cytogenetic; NA = not applicable. Abnormalities are expressed in percentages of abnormal nuclei.

the detection of chromosomal abnormalities can be performed at the single cell level in both dividing and non-dividing cells, thus circumventing the need of metaphase preparations from tumor cells. The largest series of patients studied by FISH in the literature showed that about 80% of CLL patients at diagnosis can have a chromosomal abnormality [3]. The fact that also in this study we found a similar percentage of aberrations is quite surprising, even if in a small number of patients studied, because of a limited number of probes utilized. In fact we did not use probes for chromosomes 6q and 11q, which usually account for more than 20% of chromosomal abnormalities in CLL [7–9].

This phenomenon could be due to the presence of additional clones arising in CLL patients with a disease in progression, probably representing the cause of resistance to chemotherapy and/or the rapid growth of the tumor.

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