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Acute lymphoblastic leukemias with normal karyotypes are not without genomic aberrations

Anu Usvasalo[a,*], Riikka Räty[b], Arja Harila-Saari[c], Pirjo Koistinen[d], Eeva-Riitta Savolainen[e], Kim Vettenranta[a], Sakari Knuutila[f], Erkki Elonen[b], Ulla M. Saarinen-Pihkala[a]

[a]*Hospital for Children and Adolescents, University of Helsinki, P.O. Box 281, 00029 HUS, Helsinki, Finland*
[b]*Department of Hematology, Helsinki University Central Hospital, Helsinki, Finland*
[c]*Department of Pediatrics, Oulu University Hospital, Oulu, Finland*
[d]*Department of Internal Medicine, Oulu University Hospital, Oulu, Finland*
[e]*Department of Clinical Chemistry, Oulu University Hospital, Oulu, Finland*
[f]*Department of Pathology, Haartman Institute, University of Helsinki, Helsinki, Finland*

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Abstract Current cytogenetic techniques have enabled more accurate definition of genetic aberrations in the lymphoblasts of patients with acute lymphoblastic leukemia (ALL), at least in most cases. Detecting the cryptic aberrations undetected by conventional cytogenetic methods is important for disease classification, evaluation of prognosis, and minimal residual disease follow-up. We have studied DNA copy number alterations of 27 adolescent ALL patients with normal ($n = 26$) or failed ($n = 1$) karyotype at diagnosis using microarray comparative genomic hybridization (CGH). Aberrations were detected in 85% of cases, deletions being more frequent (39 in 19 patients) than gains (14 in 10 patients). Deletions of 9p21.3 were the most common aberration, and 41% of deletions were cryptic and <5 Mb in size. We conclude that ALL without any form of genetic alteration probably does not exist. Microarray CGH is a powerful tool to reveal otherwise cryptic aberrations in adolescent ALL.

1. Introduction

Development of methods in cytogenetics has contributed to the understanding that acute lymphoblastic leukemia (ALL) is not a homogeneous disease. Structural and numerical alterations observed in the chromosomes of blast cells have enabled identification of several different ALL subgroups with unique clinical features and prognosis [1–3]. Approximately 60–70% of these changes are revealed using cytogenetic analyses with G—banding [4–7]. Development of 24-color fluorescence in situ hybridization (FISH) [8], interphase FISH with specific probes, and polymerase chain reaction (PCR) methods has improved our ability to find smaller changes and decreased the proportion of apparently normal karyotype to $<20\%$ [9].

It seems likely, however, that one or several changes in the genome are required for a blast cell to evolve into a leukemic clone, and that all cases probably harbor some form of genetic alteration. Better knowledge of the genetic alterations is essential for several reasons. Improvement in recognizing abnormalities in the blast cells will help in understanding the mechanisms that underlie leukemogenesis. More accurate delineation of genetic alterations can also provide information important for prognosis. Minimal residual disease (MRD) detection and quantification have proven important in risk-group stratification for both pediatric and adult ALL [10–15]. It has therefore become crucial that a suitable marker for MRD follow-up be identified for each ALL patient. For the majority, an MRD indicator can be found by means of flow cytometry or conventional cytomolecular genetics. Nonetheless, a small proportion of patients remains without a proper follow-up marker, and these patients would benefit from more accurate determination of their cytomolecular aberrations.

Microarray methods define and reveal novel cytogenetic changes in ALL blast cells, as has recently been shown [16–20]. In the present study, we analyzed the bone marrow samples of 27 adolescent ALL patients with normal or failed karyotype analysis. We used oligonucleotide microarray–based comparative genomic hybridization

* Corresponding author. Tel.: +358-40-5207446; fax: +358-9-47174707.
E-mail address: anu.usvasalo@helsinki.fi (A. Usvasalo).

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(CGH) to reveal previously undetected changes in the blast cell genome. The present results show that copy number alterations are common in ALL and that submicroscopic deletions comprise about one third of the aberrations. The most common aberration was deletion affecting chromosomal region 9p21.3 ($n = 11$). These cases were included in a previous report on *CDKN2A* deletions in adolescent ALL [21].

2. Material and Methods

2.1. Patients

This is a part of a larger project characterizing and evaluating ALL in adolescents and young adults. We have analyzed patients aged 10–25 years in Finland diagnosed during 1990–2007 ($n = 231$). The characteristics and outcome of this adolescent ALL population has been described in detail previously [22]. The patients were treated according to the Nordic (pediatric patients 10–16 years, $n = 133$) and Finnish Leukemia Group protocols (adult patients 17–25 years, $n = 98$). The treatment protocols have been previously described [22–24].

Of the 231 patients, 89 had normal ($n = 80$) or failed ($n = 9$) karyotype at diagnosis (32% of pediatric and 36% of adult patients, $P = 0.7$). The number of metaphases evaluated was usually ⩾20, except for two cases with 15 metaphases and two cases with only three metaphases analyzed. The DNA from initial samples was available for 27 of these 89 patients; 26 of these patients had normal karyotypes, and for the remaining 1 patient the karyotype analysis failed by G—banding at diagnosis. The key clinical characteristics (age, sex, initial white blood cell count, and immunophenotype or blast cell morphology) of the 27 patients did not differ from the rest of the patients with normal or failed karyotype (data not shown).

The study protocol was approved by the appropriate Institutional Review Boards and the National Authority for Medicolegal Affairs.

2.2. DNA isolation

Genomic DNA was extracted either from freshly frozen bone marrow ($n = 24$), or bone marrow cell pellets stored at −70°C ($n = 3$). From freshly frozen samples, DNA was isolated using a standard nonenzymatic method. For fixatives and cell pellets Puregene DNA purification kit (Gentra Systems, Minneapolis, MN) was applied. The DNA concentration was measured with a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE), and the quality of DNA was checked using agarose gel electrophoresis. Male and female reference DNAs were extracted using the standard phenol–chloroform method from pooled blood samples (four individuals) obtained from the Finnish Red Cross Blood Transfusion Service.

2.3. Hybridization

Digestion, labeling, hybridization, and data analysis of genomic DNA were performed according to the Agilent Technologies (Santa Clara, CA) protocol version 2.0 for 44 K arrays according to the manufacturer's instructions. The sample and reference DNAs, 7 μg each, were fragmented and 0.9–1.5 μg of this fragmented DNA was labeled by random priming with Cy5-dUTP and Cy3-dUTP. Labeled samples were purified, combined and hybridized to human genome CGH 44B oligo microarray slides, except for two samples which were hybridized to 244A oligo microarray slides (Agilent Technologies). The samples were hybridized against sex-matched reference DNAs. Confirmation of the array CGH results by, for example, FISH or PCR was not possible because of the lack of undamaged cells and the minimal amount of sample material. However, numerous previous reports by us and others have indicated that the oligo-based system used here is reproducible and that the method does not produce false positive results [25–27]. The methods may not reveal changes of <1 Mb or changes in mosaic form.

2.4. Data analysis

The images were analyzed and the data was extracted using Agilent Feature Extraction Software version 9.5, including dye normalization (linear lowess). For data analysis, Agilent CGH Analytics software version 3.5 was used. The starting and ending points of the aberrations were confirmed by the ADM-2 algorithm with 6.0 threshold (Agilent Technologies). Our results were compared to the Database of Genomic Variants (http://projects.tcag.ca/variation) to rule out the possibility of copy number polymorphisms.

3. Results

Of the 27 patients, 26 had initially normal karyotypes; for the remaining patient, G—banding and 24-color FISH failed and TEL/AML1 FISH yielded a normal result. FISH screening for *ETV6/RUNX1* (previously *TEL/AML1*) fusion has been done since 1998 for pediatric patients (7 patients in our study). According to flow cytometry, 8 of the 27 patients had T-cell ALL and 13 had precursor B-ALL. A mixed lineage disease was diagnosed in 5 of the 27 patients (according to the European Group for the Immunological Characterization of Leukemias (EGIL) [28]), and for the remaining patient the immunophenotype was not known. Eighteen patients had normal karyotype and no other molecular cytogenetic marker for MRD follow-up. Eleven patients (41%) were screened for immunoglobulin and/or T-cell receptor gene rearrangements and 2 (7%) for overexpression of Wilms tumor gene 1 (*WT1*). The result was positive for 9 patients for rearrangements screening and in 2 patients for WT1 overexpression.