Experimental and Molecular Pathology 101 (2016) 38–43

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Oligo-based aCGH analysis reveals cryptic unbalanced der(6)t(X;6) in pediatric t(12;21)-positive acute lymphoblastic leukemia

Eigil Kjeldsen

Hemodiagnostic Laboratory, Cancer Cytogenetics Section, Department of Hematology, Aarhus University Hospital, Tage-Hansens Gade 2, DK-8000 Aarhus C, Denmark

ARTICLE INFO

Article history:
Received 29 February 2016
and in revised form 18 May 2016
Accepted 18 May 2016
Available online 20 May 2016

Keywords:
B-ALL
ETV6-RUNX1
aCGH
Cryptic unbalanced translocation
der(6)t(X;6)

ABSTRACT

Secondary chromosomal aberrations are necessary for development of overt leukemia in t(12;21)/*ETV6-RUNX1*-positive acute lymphoblastic leukemia (ALL). Conventional cytogenetic analysis supplemented with locus-specific FISH analyses is gold standard to detect important clonal aberrations in this disease group. However, adequate chromosome banding analysis may often be hampered by poor chromosome morphology and banding patterns in pediatric ALL cases, which may hinder identification of possible clinical important additional chromosomal aberrations.

We used oligo-based high-resolution aCGH (oaCGH) analysis as an adjunct tool to enhance conventional cytogenetic analysis in pediatric acute B-cell lymphoblastic leukemia in a prospective single center study during a 4-year period (2012–2015).

In a consecutive series of 45 pediatric B-ALLs, we identified eight patients with t(12;21)/*ETV6-RUNX1* fusion by FISH analysis. In three of the patients, oaCGH analysis revealed concurrent Xq duplication and 6q deletion, which was cryptic by G-banded analysis. FISH analyses with whole chromosome painting probes confirmed the imbalances and showed an unbalanced translocation der(6)t(X;6) in all three patients. A search in the literature revealed two additional pediatric patients with cryptic der(6)t(X;6) in t(12;21)-positive ALLs. No common break points on Xq or 6q could be determined between the five patients.

This study highlights the importance of oaCGH analysis as an adjunct cytogenetic tool to detect cryptic chromosomal aberrations. Further, the study adds to understanding the full spectrum of secondary chromosomal aberrations in the very common t(12;21)-positive pediatric ALL disease group. We suggest that the unbalanced der(6)t(X;6), which is cryptic to conventional cytogenetics, is a non-random secondary event in this disease group. It might be that the specific combination of concurrent Xq duplication and 6q-deletion results in gain of possible oncogenes on Xq and loss of possible tumor suppressor genes on 6q that are important for the leukemic propagation of t(12;21)-positive hematopoietic cells in a subset of ALLs.

1. Introduction

The t(12;21)(p13;q22), leading to *ETV6/RUNX1* fusion, is the most common translocation in precursor B-cell acute lymphoblastic leukemia (ALL) (Harrison and Johansson, 2015). Although this translocation is of importance for leukemogenesis, it is not sufficient for leukemic transformation (Greaves, 2005; Hong et al., 2008). It has been shown that *ETV6/RUNX1* is present in neonatal blood spots from children who later developed ALL, but also that this translocation can be present in children who did not develop ALL at later ages (Mori et al., 2002). It is, however, an essential component that eventually promotes the acquisition of secondary aberrations, which are necessary for overt disease (Kuiper et al., 2007; Mullighan and Willman, 2011).

It has been shown that the majority of the secondary aberrations in t(12;21)-positive ALLs, particularly the drivers, occur postnatally (Bateman et al., 2010; Morak et al., 2013). The most common secondary aberrations comprise deletions of the non-translocated *ETV6* allele and genes that regulate B-cell development and differentiation (*PAX5*, *VPREB1*, *EBF1*, *IKFZ1*), proliferation (*BTG1*), and apoptosis (*BMF*) (Kawamata et al., 2008; Kuiper et al., 2007; Mangum et al., 2014; Mullighan et al., 2007, 2009; Ofverholm et al., 2013; Waanders et al., 2012). It is currently unclear when clonal diversification starts and becomes a critical component of the leukemia development (Hong et al., 2008). Although many secondary aberrations are described to be part of this diversification, their complete chromosomal nature are still elusive (Forestier et al., 2007; Harrison and Johansson, 2015).

Metaphase banding analysis is routinely performed in ALL to detect clonal aberrations, which is of great importance for prognosis and treatment strategy. Certain aberrations are, however, cryptic to banding analysis and FISH or PCR-based methods are necessary to detect these, of which the t(12;21) is one of the most recognized (Spathas et al., 1999). In pediatric ALL, poor chromosome banding and morphology is frequently observed, which may preclude identification of important

E-mail address: EIGIL.KJELDSEN@CLIN.AU.DK.

http://dx.doi.org/10.1016/j.yexmp.2016.05.010
0014-4800/

chromosomal aberrations (Betts et al., 2008). Twenty-four-color karyotyping and oligo-based high-resolution aCGH (oaCGH) analysis are technologies that can complement or enhance metaphase cytogenetics in acute leukemias (Kerndrup and Kjeldsen, 2001; Kjeldsen, 2015).

As part of a prospective population-based study of pediatric ALL, we here characterize three unbalanced cryptic translocations der(6)t(X;6) with variable chromosomal breakpoints, which was detected by oaCGH analysis in eight t(12;21)-positive ALL patients.

2. Patients and methods

We identified three t(12;21)-positive pediatric ALL cases with concurrent deletion on 6q and duplication on Xq following oaCGH analysis from a series of eight patients (5 boys and 3 girls, aged 1–9 years) with t(12;21)-positive ALL. The demographic details and clinical information about the three included patients are given in Table 1. The patients' bone marrow aspirates were examined prospectively in the period between January 2012 to December 2015 at a single cytogenetic center. The patients were from Region Midtjylland, Denmark, and in this period, 45 patients with newly diagnosed B-ALL were referred for cytogenetic analysis. All of these patients were routinely examined by G-banding, fluorescence *in situ* hybridization (FISH) and oaCGH analyses. The initial karyotypes from G-banding and FISH analyses of the three included t(12;21)-positive patients are given in Table 2. The presence of t(12;21)/*ETV6-RUNX1* fusion was established by FISH analysis of bone marrow aspiration cultures at diagnosis utilizing the LSI ETV6/RUNX1 ES dual-color translocation probe set (Abbott Molecular, Wiesbaden, Germany). In addition, the B-ALL patients were also screened with locus-specific FISH probes for aberrations involving the following genes: *CDKN2A* (9p21.3) (Abbott Molecular), *KMT2A* (previously *MLL*, 11q23) (Abbott Molecular), *TCF3* (19p13.3) (Dako Denmark A/S, Glostrup, Denmark), and *BCR* (22q11.23)/*ABL1* (9q34.12) (Abbott Molecular). All FISH probes were applied according to the manufacturer's instructions, and G-banding was done as described (Paulsson et al., 2013). Karyotypes were described according to ISCN (2013).

oaCGH analysis was done using the CytoChip Cancer 4x180K v2.0 (Illumina/BlueGnome, Cambridge, UK) according to manufacturer's instructions as described in Kjeldsen and Roug, (2012). Briefly, DNA purified from bone marrow cells was used in combination with pooled genomic DNA (Promega Biotech AB, Nacka, Sweden) as reference in a sex-matched setup. After hybridization, washing, and drying, the oligo-arrays were scanned at 2.5 μm with GenePix 4400A microarray scanner (Molecular Devices, LLC, Sunnyvale, California, USA). Initial analysis and normalization was done with BlueFuse Multi v2.6 (BlueGnome, Cambridge, UK). The normalized log2 probe signal values were imported into Nexus Copy Number software v7.5 (BioDiscovery, California, USA). FASST2 segmentation algorithm with a minimum of 3 probes/segment was used for analysis and visualization. Regions of gains and losses contained within copy number variable regions (CNVs) were discarded. Reference genome was NCBI build 37 (hg19) and the University of California Santa Cruz (UCSC) database (http://genome.ucsc.edo) was used for bioinformatics analysis.

Twenty-four-color karyotyping (24XCyte kit, MetaSystems, Altlussheim, Germany) and whole chromosome painting (Kreatech, Amsterdam, The Netherlands) were done according to the manufacturer's instructions and used to confirm the aCGH results and examine for possible structural aberrations.

Table 2
Karyotypes of the three t(12;21)-positive patients following initial G-banding and locus-specific FISH analysis at diagnosis.

Patient	Karyotype
1	46,XY[25].nuc ish (*CDKN2A*x2)[200],(*ABL1*,*BCR*)x2[200],(*KTM2A*x2)[200], (*ETV6*x2,*RUNX1*x3)(*ETV6* con *RUNX1*x1)[148/200],(*TCF3*x2)[200]
2	47,XX,+16[3]/46,XX[22].nuc ish (*CDKN2A*x1)[39/200],(*ABL1*,*BCR*)x2[200],(*KTM2A*x2)[200], (*ETV6*x2,*RUNX1*x3)(*ETV6* con *RUNX1*x1)[177/200],(*TCF3*x2)[200]
3	46,XY[25].nuc ish (*CDKN2A*x2)[200],(*ABL1*,*BCR*)x2[200],(*KTM2A*x2)[200], (*ETV6*x2,*RUNX1*x3)(*ETV6* con *RUNX1*x1)[155/200],(*TCF3*x2)[200]

3. Results

Within the series of eight pediatric *ETV6/RUNX1*-positive cases, we detected concurrent 6q deletions and Xq duplications in three patients by oaCGH analysis (Fig. 1). Apart from these non-random genomic imbalances, eight additional aberrations were detected including major imbalances at 9p, 12p, 13q, and chromosome 16 (Table 3). A total of 14 genomic imbalances were detected in this group of patients giving an average of 4.7 imbalances per case. Deletions involving the genes *EBF1*, *ETV6*, *PAX5*, *BTG1*, *VPREB1*, and *IKZF1* are frequent secondary aberrations in *ETV6/RUNX1*-positive ALLs, but we only observed deletions in Patient 3 affecting the *EBF1*, *ETV6*, and *VPREB1* genes. In Patient 2, we detected deletion of the *VPREB1* gene.

Comparing the oaCGH findings (Table 3) with the findings by G-banding and locus-specific FISH analyses (Table 2), it was only the trisomy 16 and del(9)(p21.3p21.3) in Patient 2 that was detected by conventional karyotyping and FISH analysis, respectively. In Patient 3, however, the deletion on 12p13.2-p11.1 is centromeric to the genomic position of the ETV6 FISH probe and will not be detected by FISH.

To examine why G-banding analysis missed the large genomic aberrations at 6q and Xq, we performed 24-color karyotyping and/or chromosome painting with whole chromosome painting probes for chromosomes X and 6 in a dual-color setup (Fig. 1). In all three patients,

Table 1
Clinical information of the included t(12;21)-positive precursor B-ALL patients.

	Patient 1	Patient 2	Patient 3
Sex/age (year, month)	Male/2,7	Female/4,7	Male/9,3
WBC (10^9/l)	14.3	2.6	4.5
Platelets (10^9/l)	131	76	36
Hgb (mM)	4.3	5.0	3.5
LDH (U/l)	518	350	540
Immunophenotype	85% immature B-lymphocytes in BM: CD10++, CD19+, CD20-, CD34+	69% immature B-lymphocytes in BM: CD10++, CD19+, CD20-, CD34+	45% immature B-lymphocytes in BM: CD10++, CD19+, CD20-, CD34+
Risk assignment:			
–At diagnosis	SR	SR	SR
–MRD-level at day 29	0.02% => SR	0.03% => SR	0.03% => SR
Therapy	SR NOPHO Protocol 2008	SR NOPHO Protocol 2008	SR NOPHO Protocol 2008
Survival	Alive, 32 mo. EFS	Alive, 28 mo. EFS	Alive, 20 mo. EFS

WBC: total white blood cell counts; Hgb: hemoglobin; MRD: minimal residual disease; SR: standard risk; EFS: event free survival; BM: bone marrow; LDH: P-lactatedehydrogenase; NOPHO: Nordic Society of Paediatric Haematology and Oncology.