Gene 595 (2016) 39–48

Contents lists available at ScienceDirect

Gene

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

Research paper

Characterization of a novel acquired der(1)del(1)(p13p31)t(1;15)(q42;q15) in a high risk t(12;21)-positive acute lymphoblastic leukemia

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ARTICLE INFO

Article history:
Received 9 August 2016
Received in revised form 15 September 2016
Accepted 20 September 2016
Available online 21 September 2016

Keywords:
High-risk B-ALL
MRD
ETV6-RUNX1
Unbalanced translocation
aCGH
Interstitial deletions

ABSTRACT

The t(12;21)(p13;q22) with *ETV6-RUNX1* fusion occurs in 25% of cases of B-cell precursor acute lymphoblastic leukemia (BCP-ALL); and is generally associated with favorable prognosis. However, 15–20% of the t(12;21)-positive cases are associated with high-risk disease due to for example slow early responses to therapy. It is well-known that development of overt leukemia in t(12;21)-positive ALL requires secondary chromosomal aberrations although the full spectrum of these cytogenetic alterations is yet unsettled, and also, how they may be associated with disease outcome. This report describes the case of an adolescent male with t(12;21)-positive ALL who displayed a G-banded karyotype initially interpreted as del(1)(p22p13) and del(15)(q15). The patient was treated according to NOPHO standard risk protocol at diagnosis, but had minimal residual disease (MRD) at 6,4% on day 29 as determined by flow cytometric immunophenotyping. Because of MRD level > 0.1% he was then assigned as a high risk patient and received intensified chemotherapy accordingly. Further molecular cytogenetic studies and oligo-based aCGH (oaCGH) analysis characterized the acquired complex structural rearrangements on chromosomes 1 and 15, which can be described as der(1)del(1)(p13.1p31.1)t(1;15)(q42;q15) with concurrent deletions at 1q31.2-q31.3, 1q42.12-q43, and 15q15.1-q15.3. The unbalanced complex rearrangements have not been described previously. Extended locus-specific FISH analyses showed that the three deletions were on the same chromosome 1 homologue that was involved in the t(1;15), and that the deletion on chromosome 15 also was on the same chromosome 15 homologue as involved in the t(1;15). Together these findings show the great importance of the combined usage of molecular cytogenetic analyses and oaCGH analysis to enhance characterization of apparently simple G-banded karyotypes, and to provide a more complete spectrum of secondary chromosomal aberrations in high risk t(12;21)-positive BCP-ALLs.

1. Introduction

The t(12;21)(p13;q22), leading to *ETV6*/*RUNX1* fusion, is exclusively associated with B-lineage ALL (Harrison and Johansson 2015). It is the most common primary acquired genetic aberration in pediatric B-cell precursor acute lymphoblastic leukemia (BCP-ALL) occurring in 19 to 32% of childhood ALL (Borkhardt et al. 1997; Borowitz et al. 1998) but are rare in adult ALLs being present in <4% of adult cases (Shurtleff et al. 1995; Aguiar et al. 1996; Kwong and Wong 1997). The peak incidence of t(12;21)-positive BCP-ALL is between the ages 3–6 years (Golub et al. 1995; Romana et al. 1995). BCP-ALL with the *ETV6*/*RUNX1* fusion has a very favorable clinical outcome (Harrison and Johansson 2015).

A stepwise clonal evolution model has been suggested in t(12;21)-positive ALL where the *ETV6*/*RUNX1* fusion constitute the initiating event transforming a normal cell in utero into a pre-leukemic clone (Greaves 2005; Hong et al. 2008). This view is supported by several observations: 1) retrospective studies have shown that the fusion gene can be detected in the blood of children long before presentation of overt leukemia (Mori et al. 2002); 2) comparative genomic studies of monozygotic twins with concordant ALL revealed that whilst the *ETV6*/*RUNX1* fusions are identical, all other recurrent secondary genetic changes are distinctive (Bateman et al. 2010; Ma et al. 2013); and 3) in discordant monozygotic twins, it was shown that a healthy co-twin carries the putative pre-leukemic clone with *ETV6*/*RUNX1* fusion but without secondary genetic changes present in the co-twin with ALL (Hong et al. 2008; Bateman et al. 2010). Together these data indicate that *ETV6*/*RUNX1*-positive ALL is generated through a multi-step mechanism with accumulation of necessary additional genetic changes post-natally for overt leukemia to develop (Hong et al. 2008; Bateman et al. 2010; van der Weyden et al. 2011; Morak et al. 2013).

Abbreviations: BAC, bacterial artificial chromosome; BCP-ALL, B-cell precursor acute lymphoblastic leukemia; FISH, fluorescence in situ hybridization; MRD, minimal residual disease; oaCGH, oligo-based array comparative genomic hybridization.

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http://dx.doi.org/10.1016/j.gene.2016.09.030
0378-1119/

To fully understand the genetic evolution of BCP-ALL identification of the complete spectrum of genetic changes that accompany the *ETV6/RUNX1* fusion gene is necessary. Furthermore, critical pathogenetic insights may be gained from studying the correlation pattern of the different chromosomal aberrations. Several techniques have been deployed to screen and study chromosomal aberrations in ALL. Routine cytogenetic diagnostics of ALL include chromosome banding analysis of at least 20 metaphases for classification of specific leukemia. Complementary techniques such as fluorescence in situ hybridization (FISH), and reverse transcriptase-polymerase chain reaction (RT-PCR) can be used to detect the cryptic t(12;21)(p13;q22) (Harbott et al. 1997). Locus-specific FISH analysis is useful to identify specific translocations, but is limited to the type of probe used to investigate the genomic region of interest, and is not genome-wide. Although 24-color karyotyping is a genome-wide FISH analysis it is hampered by its requirement for metaphases and its limited resolution (Kjeldsen and Kølvraa 2002; Anderson 2010) it has been used to detect additional cryptic aberrations in t(12;21) positive ALLs, (Betts et al. 2008; Stanchescu et al. 2009; Kjeldsen 2016). Microarrays have also been applied to study copy number alterations (CNAs) (Kuiper et al. 2007; Mullighan et al. 2007; Tsuzuki et al. 2007; Kawamata et al. 2008; Zakaria et al. 2012; Kjeldsen 2015) and expression signatures (Yeoh et al. 2002) in patients with ALL.

Many pathogenic chromosomal abnormalities have been reported in ALL patients using the high resolution array comparative genomic hybridization (aCGH) platform and single nucleotide polymorphism (SNP) arrays. The most highly recurrent secondary genetic aberrations comprise deletions of genes that regulate B-cell development and differentiation (*PAX5*, *VPREB1*, *EBF1*, *IKFZ1*), proliferation (*BTG1*), cell cycle (*CDKN2A* and *CDKN2B*) and apoptosis (*BMF*) (Kuiper et al. 2007; Mullighan et al. 2007; Kawamata et al. 2008; Mullighan et al. 2009; Waanders et al. 2012; Ofverholm et al. 2013; Mangum et al. 2014). In a recent study of 57 cases, necessary additional genetic events in ETV6-RUNX1 positive ALL were characterized using paired end and exomic sequencing (Papaemmanuil et al. 2014). In this study it was shown that the total mutation load was relatively small and that two mutational signatures could be identified. The most recurrent secondary genetic events were CNAs, most of which were deletions where the break points have complete or partial nonamer-heptamer RAG recognition motifs. It was suggested by these findings that the critical secondary events may be instigated by off-target RAG activity. The other mutational signature was single nucleotide variants (SNVs), which were less recurrent, being transitions or transversions at cytosines indicating a role of APOBEC as observed in other cancers (Roberts et al. 2013).

In contrast to high-risk subtypes of childhood ALL (Roberts et al. 2012), *ETV6/RUNX1*-positive ALL have very few mutations impinging on kinase pathways, which may help to explain its generally very good prognosis. However, approximately 15% of the ETV6/RUNX1 positive patients have high-risk disease, which because of their relative rarity are less well characterized genetically (Forestier et al. 2008). High-risk patients may have high WBC counts or a slow early response, defined either as bone marrow (BM) minimal residual disease (MRD) ≥0.1% at day 29 of induction therapy or BM blasts ≥5% by morphology at day 15 of induction therapy, and requires intensified chemotherapy (Borowitz et al. 2015). In addition, secondary cytogenetic aberrations such as complex karyotypes, extra copy of der(21)t(12;21), gain of one or two RUNX1 alleles, or deletion of second ETV6 allele have been associated with poor prognosis (Attarbaschi et al. 2004; Peter et al. 2009).

Here we characterized a high-risk t(12;21)-positive BCP-ALL in an adolescent male. He was initially stratified as standard risk, but because of a slow early response at day 29 he was re-stratified as high-risk. Initial G-banding was interpreted as del(1)(p22p13) and del(15)(q15), whereas further molecular cytogenetic studies and oligo-based aCGH (oaCGH) analysis revealed acquired complex structural rearrangements involving chromosomes 1 and 15, which can be described as der(1)del(1)(p13.1p31.1)t(1;15)(q42;q15) with concurrent deletions at 1q31.2-q31.3, 1q42.12-q43, and 15q15.1-q15.3; genomic alterations that have not been described before. This report shows the great importance of oaCGH analysis to enhance cytogenetic diagnostics in BCP-ALL, and adds to defining the full spectrum of secondary cytogenetics events in t(12;21)-positive ALL, which may be of importance for leukemogenesis, prognosis and treatment.

2. Material and methods

2.1. Clinical description

A 19-years old previously well male was admitted to Department of Hematology, Aarhus University Hospital, with a history of anemia, coagulopathy, and flu-like symptoms three weeks prior to admission. The patient had sore throat and dysphagia, and was prior to admission treated with antibiotics for 10 days without effect. One week before admission there were several episodes of epistaxis. On admission the patient showed pallor, petechiae, and microadenopathy. Results from examination of peripheral blood and bone marrow (BM) are summarized in Table 1. FISH analysis with ETV6/RUNX1 probes demonstrated the presence of *ETV6/RUNX1* fusion gene. The patient was diagnosed with t(12;21)-positive precursor B-ALL and treated according to the Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL protocol as standard risk. However, minimal residual disease (MRD) determined by flow cytometry (FCM) at day 29 revealed 6.4% residual blasts. Because of MRD level > 0.1% he was re-stratified to the high-risk group, and treated according to the NOPHO high risk ALL protocol. He obtained complete hematological remission (CR) and MRD measurement at day 69 by flow cytometry showed 0.0128% residual blasts. During the intensified treatment he suffered *Staphylococcus aureus* sepsis and abscesses in his lower extremities, which were treated with antibiotics and surgical drainage. A hematopoietic stem cell transplantation (HSCT) was planned in CR1 because of his slow early treatment response. However, this has been postponed due to severely impaired lung diffusion capacity at 40%. Lung perfusion scintigrahy revealed subsegment lung embolisms, which are being treated with anticoagulantia. Echocardiography was normal. At present, 15 months after primary diagnosis, he is still in CR1 while receiving chemotherapy, and awaits HSCT as soon as his lung function improves.

Table 1
Clinical information on the t(12;21)-positive BCP-ALL.

	Patient
Sex/age (year, month)	Male/19,9
WBC (10^9/l)	62
Platelets (10^9/l)	12
Hgb (mM)	3.5
LDH (U/l)	1865
Immunophenotype	75% immature B-lymphocytes in BM: CD10++, CD13+, CD19++, CD20+, CD34−
Risk assignment:	
- At diagnosis	SR
- MRD-level at day 29	6.4% (FCM) ≥ HR
MRD-level at day 69	0.0128% (FCM)
Initial therapy	SR NOPHO Protocol 2008
Survival	Alive 15 months after Dx

WBC: total white blood cell counts; Hgb: hemoglobin; MRD: minimal residual disease; SR: standard risk; HR: high risk; BM: bone marrow; LDH: P-lactate-dehydrogenase; NOPHO: Nordic Society of Paediatric Haematology and Oncology; FCM: Flow cytometry measurement.