Homozygous deletions of CDKN2A are present in all dic(9;20) (p13.2;q11.2)-positive B-cell precursor acute lymphoblastic leukaemias and may be important for leukaemic transformation

The dic(9;20)(p13.2;q11.2) is a recurrent chromosomal aberration present in up to 5% of childhood B-cell precursor acute lymphoblastic leukaemia (BCP ALL) patients and associated with worse survival compared with the standard risk aberrations t(12;21)(p13;q22) and high hyperdiploidy (Zachariadis et al, 2011). Molecular studies have revealed scattered breakpoints on both chromosomes, however all are clustered within 9p13-2 and 20q11-2 (Schoumans et al, 2006; An et al, 2009). In some cases, dic(9;20) has been shown to rearrange the PAX5 gene with various partners at 20q (An et al, 2008), but in most instances the functional outcome is unknown and possibly heterogeneous.

CDKN2A deletions at 9p21-3 are among the most frequent aberrations in childhood BCP ALL, and can be detected by single nucleotide polymorphism (SNP array) in 21–34% of cases (Mullighan et al, 2007; Sulong et al, 2009). In dic(9;20)-positive BCP ALL, one allele of the CDKN2A locus is always lost because it is located distally to the 9p13-2 breakpoint (Schoumans et al, 2006; An et al, 2009). Using interphase fluorescence in situ hybridization (FISH) analyses, homozygous CDKN2A deletions in dic(9;20) have been reported in about one-third of cases.

To evaluate further the presence of genomic imbalances in this specific subgroup, we analysed diagnostic samples from six dic(9;20)-positive paediatric BCP ALL patients by SNP array (Human610W-Quad v.1.0; Illumina Inc, San Diego, CA, USA), according to the manufacturer’s instructions (Infinium HD Assay Super manual, #11322427 rev C). Analysis was done using Illumina Genome Studio (Illumina Inc) and Nexus Copy Number (BioDiscovery Inc, El Segundo, CA, USA). FISH analysis for dic(9;20) was performed as previously described (Zachariadis et al, 2011). The study was approved by the research ethics committee at Karolinska Institutet and informed consent was obtained in accordance with the Declaration of Helsinki.

As expected, considering the unbalanced nature of the dic (9;20), the SNP array analysis revealed loss of the p arm of chromosome 9 and the q arm of chromosome 20 in all six patients (Fig 1A). In three cases (Patients 1, 3, and 5), the breakpoints on chromosome 9 were present within the PAX5 gene, in introns 7, 8, and 5 respectively, whereas Patient 6 had a breakpoint 200 kb closer to the centromere, within ZCCHC7, and Patients 2 and 4 had breakpoints an additional 2 Mb away (Table I). On chromosome 20, the three cases with breakpoints within PAX5 all had breakpoints within C2orf112 intron 2 (however probe density in this region was low), previously shown to be fused in-frame with PAX5 in dic(9;20)-positive cases (An et al, 2008; Kawamata

References


et al, 2008). Apart from homozygous CDKN2A deletions (see below) and rearrangements involving the TRG, HLA and IGL loci, no other imbalance was recurrent (Table I).

Interestingly, all six patients analysed had homozygous deletions of CKDN2A. The deletions displayed a pattern of one large, terminal deletion, as expected in dic(9;20), and one small, interstitial deletion. In contrast, previous interphase FISH analyses of these cases revealed only hemizygous loss of the CDKN2A gene (Zachariadis et al, 2011). The sizes of the homozygous deletions, as ascertained by the SNP array, ranged from 23 to 201 kb and would thus have escaped detection by FISH analysis (Table I; Fig 1B). It is...
Noteworthy that the proximal breakpoints of the CDKN2A deletions were identical in all six cases (Illumina probe rs2069422; Fig 1B). Two cases (Patients 3 and 6) also shared distal breakpoints (rs756641), rendering their CDKN2A deletions fully synonymous. This strongly indicates a common underlying mechanism, such as illegitimate V(D)J recombination mediated by the recombination-activating gene complex acting on ectopic recombination signal sequences, previously shown to be frequently associated with CDKN2A deletions in lymphoid leukaemias (Kitagawa et al., 2002).

Patient 1 initially presented with a severe varicella zoster virus infection and neutropenia. Bone marrow (BM) analysis revealed an indeterminate lymphoid clone, and a normal karyotype. The blood parameters improved for 3 weeks, after which the patient was re-admitted due to a grossly increasing white blood cell count, suggesting overt leukaemia. A second BM sample revealed dic(9;20)-positive BCP ALL. FISH analysis on cells from the 'pre-leukaemic' sample confirmed the presence of dic(9;20) in 38% of interphase nuclei but not on metaphases. Most interestingly, the homozygous loss of CDKN2A was not present in the 'pre-leukaemic' sample collected 3 weeks prior to overt leukaemia, but was present at the diagnosis of ALL (Patient 1b and 1a respectively; Fig 1C).

Table I. Clinical, cytogenetic, and SNP array data for six dic(9;20)-positive BCP ALL cases.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Karyotype</th>
<th>Breakpoint position*</th>
<th>CDKN2A status</th>
<th>Additional aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chr 9</td>
<td>Chr 20</td>
<td>Deletion type</td>
</tr>
<tr>
<td>1a†</td>
<td>F</td>
<td>1</td>
<td>46,XX[27]</td>
<td>36 884 656</td>
<td>30 512 043</td>
<td>Hemizygous</td>
</tr>
<tr>
<td>1b‡</td>
<td>F</td>
<td>1</td>
<td>45,XX dic(9;20)(p13;2;q11-2)[5]/46,XX[9]</td>
<td>36 884 656</td>
<td>30 512 043</td>
<td>Homozygous</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>3</td>
<td>46,XX dic(9;20)(p13;2;q11-2),+21[18]/46,XX[8]</td>
<td>38 781 694</td>
<td>30 116 038</td>
<td>Homozygous</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>1</td>
<td>46,XX[24].ish, dic(9;20)(p13-2q11-2)</td>
<td>36 846 268</td>
<td>30 512 043</td>
<td>Homozygous</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>2</td>
<td>46,XX.t(7;22)(p13;q11), dic(9;20)(p13-2q11-2)[5]/46,XX[6]</td>
<td>38 781 694</td>
<td>29 297 270</td>
<td>Homozygous</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>1</td>
<td>45,XX dic(9;20)(p13;2;q11-2)[22]/46,XX[1]</td>
<td>36 975 404</td>
<td>30 512 043</td>
<td>Homozygous</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>8</td>
<td>46,XY dic(9;20)(p13;2;q11-2),+8,+21[10]/47, idem,+mar[11]</td>
<td>37 169 379</td>
<td>30 732 585</td>
<td>Homozygous</td>
</tr>
</tbody>
</table>

Patients 1b and 2–6 previously published (Schoumans et al., 2006; Zachariadis et al., 2011).

ish, in situ hybridization; FISH, fluorescence in situ hybridization.

*Reference build hg18 (http://genome.ucsc.edu/cgi-bin/hgGateway?hgId=289071031&clade=mammal&org=Human&db=hg18).
†‘Pre-leukaemic’ sample.
‡Diagnostic sample.
CDKN2A deletions in ALL with dic(9;20). In this patient, BM samples were available both at a ‘pre-leukaemic’ stage and at the time of overt leukaemia, in both instances having the dic(9;20); a homozygous CDKN2A deletion was, however, only found at the latter time point. Thus, there was a progression from a hemizygous to a homozygous deletion of CDKN2A during leukaemic transformation of the dic(9;20)-positive clone. In this context it is worthy of note that preferential expansion of CDKN2A-deleted clones at relapse has previously been reported (Mullighan et al., 2008). However, further studies on the impact of homozygous CDKN2A deletions in dic(9;20)-positive BCP ALL and the relationship between ancestral, non-leukaemic dic(9;20)-harbouring clones and CDKN2A deletions are warranted.

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Authorship and disclosures

VZ performed research, analysed data and wrote the paper. JS, GB, MN analysed data. MH, EF analysed data and provided clinical data. BJ analysed data and wrote the paper. AN, principal investigator, designed research, analysed data and wrote the paper. The authors declare no conflict of interest.

References


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