Nondisjunction of chromosomes leading to hyperdiploid childhood B-cell precursor acute lymphoblastic leukemia is an early event during leukemogenesis

E. Renate Panzer-Grümayr, Karin Fasching, Simon Panzer, Klaudia Hettinger, Klaus Schmitt, Sylvia Stöckler-Ipsiroglu, and Oskar A. Haas

A hyperdiploid karyotype is found in 30% of B-cell precursor acute lymphoblastic leukemias in childhood. The time of nondisjunction of chromosomes leading to hyperdiploidy during leukemogenesis is unknown. We used the 3 clonotypic immunoglobulin heavy chain (IgH) gene rearrangements as molecular markers for each of the 3 chromosomes 14 in a case with hyperdiploid acute lymphoblastic leukemia to define the order of events—namely, somatic recombination and nondisjunction of chromosomes—during leukemia development. A partial sequence homology of the incomplete DJH rearrangement with 1 of the 2 nonfunctional VDJH rearrangements suggests that the doubling of chromosomes had occurred after this DJH rearrangement and thus during early B-cell differentiation. The occurrence of the nondisjunction of chromosomes as well as ongoing rearrangement processes in utero were confirmed by the presence of all 3 IgH rearrangements in neonatal blood spots, providing the first evidence that hyperdiploidy formation is an early event in leukemogenesis in these leukemias.

Patient, materials, and methods

Patient

We selected one hyperdiploid BCP ALL (CD10+, CD19−, cμ−) case with a trisomy 14 and 3 IgH rearrangements, a 2.6-year-old boy. Hyperdiploidy was identified by routine cytogenetics in bone marrow cells at diagnosis. The presence of trisomy 14 was confirmed by fluorescence in situ hybridization in 76% of cultured interphase cells. The study was approved by the ethics committee of the Children’s Cancer Research Institute, St Anna Kinderspital, and informed consent was obtained from the parents.

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Figure 1. Analysis of leukemia-specific IgH rearrangements in single leukemic cells and neonatal blood spots of a patient with hyperdiploid ALL.

Table 1. Nucleotide sequences of the clonotypic IgH rearrangements in a hyperdiploid ALL

<table>
<thead>
<tr>
<th>Rearrangements</th>
<th>V</th>
<th>N</th>
<th>D</th>
<th>J</th>
<th>Gene segments</th>
<th>Open reading frame</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td></td>
<td></td>
<td>g</td>
<td></td>
<td>D3-22/J5</td>
<td></td>
</tr>
<tr>
<td>ii</td>
<td>V3</td>
<td>aag</td>
<td>– 8</td>
<td></td>
<td>D3-22/J5</td>
<td></td>
</tr>
<tr>
<td>iii</td>
<td>V3</td>
<td>gat</td>
<td>– 3</td>
<td></td>
<td>D3-22/J5</td>
<td></td>
</tr>
</tbody>
</table>

Trimming of the rearranged segments is indicated by the numbers of nucleotides adjacent to D or J segments; underlined sequences indicate homology between rearrangements.

DNA preparation from fresh cells and Guthrie cards

High molecular weight DNA was extracted by proteinase K digestion followed by phenol chloroform extraction. Guthrie spots were routinely cut into 3 pieces, and DNA was extracted from each piece separately at different occasions. DNA from Guthrie cards was extracted using the DNAzol kit (Vienna Labs, Vienna, Austria) as described previously.\(^8\) Integrity of the DNA was confirmed by amplification of the exon 16 of the RET proto-oncogene.\(^9\)

Single-cell sorts

Single leukemic cells were sorted on a FACStar Plus (BD Bioscience, San Jose, CA) using the light scatter profile of the mononuclear cells from bone marrow at diagnosis, which contained more than 95% blasts. Cells were sorted in a 200-μL Eppendorf tube containing 20 μL 1× buffer and stored at −20°C until further use.

Determination of leukemia clone-specific IgH gene rearrangements

Polymerase chain reaction (PCR) amplification of incomplete and complete IgH rearrangements was performed using family-specific D and V \(\text{H}\) primers, respectively, and one J \(\text{H}\) consensus primer, as described previously.\(^9\) Amplified products were directly sequenced in both directions, and corresponding PCR products were size fractionated on 2.5% agarose (A) or 4% to 12% polyacrylamide gels and visualized by ethidium bromide staining.

Clone-specific PCR

A first-round PCR was performed using D3 and V3 family-specific primers as described above. One microliter of a 1:20 dilution of the first-round 50 μL PCR reaction was used for a second-round nested PCR. For the D3-22/J5 rearrangement, the primers were positioned into the intron region 5’ of the D3-22 segment overlapping the 3’ end of the D3 primer to avoid coamplification of the V3/D3-22/J5 rearrangement. For the 2 different complete rearrangements, 3’ primers were designed homologous to the individual VND regions and amplified with internal V3 specific primers, respectively. PCR products were size-separated on agarose and polyacrylamide gels and visualized by ethidium bromide staining. Precautions to avoid contamination with amplified material were followed as previously described.\(^8\) Amplifications were performed on a PTC 300 Thermocycler (Techne, Cambridge, United Kingdom). All PCR products of the expected size were directly sequenced after gel purification using QIAEX II Gel Extraction kit (Qiagen, Valencia, CA).

Results and discussion

The hyperdiploid leukemia with a trisomy 14 selected for our study has 3 different nonfunctional IgH rearrangements (Table 1). It can be assumed that each of these IgH rearrangements is located on a different chromosome 14. To confirm that all 3 alleles are contained within a single leukemia clone, we sorted individual leukemic cells and performed the clone-specific nested PCR for each IgH rearrangement in 10 sorted cells each. We obtained PCR products of the expected size for rearrangements A, B, and C in 9, 10, and 7 cells, respectively (Figure 1). Sequencing of these obtained ampliﬁcons revealed the respective rearrangements, thus conﬁrming monoclonality of the leukemia. Two of these rearrangements—the incomplete DJ3 and one of the complete VDJ3 rearrangements—were found to have homologous DJ3 joinings (Table 1). Thus, the chromosome harboring the original DJ3 rearrangement must have duplicated before the subsequent joining of a V3 segment to one of these incomplete rearrangements.\(^10\) These findings suggest that an incomplete DJ3 rearrangement on one allele and presumably a nonfunctional VDJ3 rearrangement on the second allele were
present in the diploid cell before mitosis, leading to the assumption that the nondisjunction of chromosome 14 is an early event in B-cell differentiation. Whereas in mice clonal expansion at an early stage of B-cell development does not require pre–B-cell receptor formation, IgH expression is a prerequisite for initiating early cell division in normal B lymphocytes in humans. Thus, an earlier transforming event—preceeding the abnormal mitosis—was necessary for this diploid cell to enter the cell cycle.

We and others have shown the prenatal origin of leukemia-specific chromosomal translocations in children with ALL by retrospective evaluation of neonatal blood spots. Using this source of the earliest available hematopoietic cells in this patient for the retrospective analysis of clonotypic IgH rearrangements, we addressed the question of whether nondisjunction leading to hyperdiploidy had occurred prenatally. Indeed, all 3 rearrangements were present already at birth (Figure 1), indicating that doubling of chromosome 14 as well as the ongoing rearrangement processes occur early during leukemogenesis, while the leukemia became clinically apparent only 2.6 years later. Because it was shown that hyperdiploidy results from one single abnormal mitosis, nondisjunction of chromosome 14 is likely to concur with that of the other multiplied chromosomes present in a hyperdiploid leukemia. However, the causes for nondisjunction as well as the impact of hyperdiploidy on leukemia development still remain elusive.

References

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