Amplification of \textit{AML1} gene in association with karyotype, age and diagnosis in acute leukemia patients*

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Received May 11, 2005

The \textit{AML1} gene, located in the chromosomal band 21q22, belongs to the runt domain family of genes and encodes the subunit of the core-binding factor (CBF). AML1 is normally expressed in all hematopoietic lineages and is essential for the transcriptional regulation of a number of hematopoietic specific genes. In acute leukemia three types of abnormality of \textit{AML1} have been observed – chromosomal translocations, point mutation and duplication or amplification of the unrearranged gene. The most common origin of extra copies of the \textit{AML1} gene is polysoy of chromosome 21 or a partial duplication of the long arm of chromosome 21, less frequently ring, isochromosome or the tandem repetition of chromosome 21.

In the study 13 children and 5 adults with ALL and AML or MDS, respectively, have been included. Using standard G-banding and dual color FISH analyses, gain of \textit{AML1} originated in polysoy of chromosome 21 in each group of patients was proved. True high-level amplification was not observed but some uncommon changes in noteworthy association with other chromosome aberrations, age or diagnoses were presented.

Key words: \textit{AML1} gene, amplification, ALL, AML

The \textit{AML1} (CBFA2, \textit{RUNXI}) gene located in the chromosomal band 21q22, belongs to a recently identified gene family of transcription factors sharing a highly evolutionarily conserved region of 128 amino acids termed the runt domain (\textit{drosophila} segmentation gene \textit{runt}, human genes \textit{AML1}, \textit{AML2}, \textit{AML3}, mouse homologues \textit{PEBF2αβ}, \textit{CBFα3}, \textit{PEPB2αA}) [1].

The \textit{AML1} gene encodes the DNA-binding subunit of the heterodimering transcriptional factor complex, core-binding factor (CBF). \textit{AML1-CBFβ} complex binds a consensus DNA core motif known as the Runt domain binding element (TGTGGT), present in transcriptional regulatory regions of genes essential for myelopoiesis [2, 3].

\textit{AML1} is normally expressed in all hematopoietic lineages and is essential for the transcriptional regulation of a number of hematopoietic specific genes, including those for granulocyte-macrophage colony stimulating factor (GM-CSF), M-CSF receptor, myeloperoxidase, neutrophil elastase, interleukin 3 (IL-3), and each of the subunits of the T-cell antigen receptor genes. Its key regulatory role in hematopoiesis has been proved on murine model. Mice lacking either \textit{AML1} or \textit{CBFβ} are defective in definitive hematopoiesis of all lineages [2, 3, 4].

In acute leukemia three types of abnormality of \textit{AML1} have been observed – chromosomal translocations with formation of new chimeric oncogene, point mutation of \textit{AML1} and duplication or amplification of the unrearranged gene [5, 6].

More than 40 different patterns of translocations or rearrangements involving the 21q22 region have been described [7]. The \textit{AML1} gene is a target of many common translocations seen in different types of leukemia, such as t(12;21) with \textit{TEL/AML1} fusion in childhood acute lymphoblastic leukemia (ALL), t(8;21) and \textit{AML1/ETO} in acute myeloid leukemia (AML) or recombination of \textit{AML1} with genes \textit{EVII}, \textit{EAP} and \textit{MDSI} on 3q26 in myelodysplasia (MDS) and in the blast phase of chronic myeloid leukemia (CML) [4, 8, 9, 10, 11].
Mutation within the AML1 gene has been identified in de novo myelodysplasia and acute myeloid leukemia, and in a therapy related to both MDS (t-MDS) and t-AML, respectively, induced by alkylating agents. Congenital mutations in AML1 caused the rare autosomal dominant disease, familial platelet disorder (FDA) with predisposition to the development of AML [3, 4, 5, 10].

Genomic amplification is a frequent genetic abnormality especially in solid tumors and many oncogenes could be activated in this way. The situation in hematological malignancies appears to be different. Rather than two known forms of amplified genes – extended chromosomes named homogeneously staining regions (HSR) or extra chromosomal elements, called double minute (dmin) chromosomes [4], in acute leukemia the most common origin of extra copies of the AML1 gene is polysomy of chromosome 21 or a partial duplication of the long arm of chromosome 21, dup(21) [7, 12, 13]. Another, infrequent chromosome aberrations can lead to gain of this gene, such as the amplification caused by ring 21 or by isochromosome, or less frequently by the tandem repetition of chromosome 21 [14]. Albeit polysomy of chromosome 21 or its long arm is usually well demonstrable by conventional cytogenetics (G-banding), a small intrachromosomal amplification can be covered up on cytogenetically unchanged chromosome 21 or by the marker chromosomes occurred in complex karyotype. Therefore, FISH techniques are especially useful for identifying chromosomal aberrations, as well as the identification of marker chromosomes [5, 7, 11, 15].

Material and methods

Patients. Bone marrow samples from 13 children of age ≤15 years, with median age of 5 years (range 2–12) and 5 adults (age >15 years) with a median age of 53 years (range 30–74) were analyzed at the time of diagnosis. None of the 18 patients had Down’s syndrome. The leukemia immunophenotype was determined by standard immunofluorescence analysis using a panel of monoclonal antibodies.

Methods. Standard chromosomes G-banding analysis was performed on mitotic bone marrow cells after short-term culture. Karyotypes were interpreted according to the International System for Cytogenetic Nomenclature (ISCN, 1995) [16].

Interphase FISH screening was routinely carried out on all children ALL patients for the prognostically significant abnormalities, TEL/AML1, BCR/ABL and rearrangements of the MLL gene. FISH identification of AML1 aberrations in cases with AML or other diagnoses were realized according to the cytogenetic finding.

FISH analyses were performed on the fixed cell suspensions used for cytogenetic analysis. Either by locus-specific TEL/AML1 or AML1/ETO probe (Vysis), and/or various whole-chromosome and centromere-specific probes were used according to the manufacturer instructions.

Results

In a group of 13 childhood patients was predominance of boys (8) over girls (5). In 12 cases the diagnosis of common ALL (cALL) and one case of AML was established. Interphase FISH screening analysis by using TEL/AML1 probe was carried out in all of children. In only 5 cases it was possible to obtain the karyotype (Tab. 1). Four children displayed hyperdiploidy (52–63 chromosomes), but the number of AML1 signals by FISH was in agreement with the number of chromosomes 21 only in one case (patient 5). The localization of AML1 extra copies was not known in other three children (2, 3, 6). The sole trisomy of 21 chromosome alike with one additional signal was obtained in patient 12. Only one child patient with AML had one normal but two derived chromosome 21; one small acrocentric chromosome dup(21)(q?) and one as a part of bigger ring chromosome confirmed by FISH could be found (Fig. 1).

In the group of adult patients dominance of 4 AML cases over the one pre-B ALL was observed. Karyotype of all patients was acquired. Three cases (14, 16, 17) presented trisomy of chromosome 21 associated with another numerical or structural changes, confirmed by FISH (Tab. 2). Patient 15 displayed near tetraploidy with four chromosomes 21 but 4–6 AML1 signals by FISH. The place of extra copies of AML1 gene was not practicable to identify due to the multiple marker chromosomes. Due to failure in preparing initial karyotype, FISH with panel of probes for childhood ALL was performed in patient 18. Surprisingly, in connection with the age of this patient, TEL/AML1 double fusion accompanying with loss of normal TEL and gain of AML1 was uncovered (Fig. 2).

Discussion

This study presents a series of 18 patients with an abnormal number of AML1 gene in children and adults with differ-
ent type of leukemia. In concordance with literature a predominance of both, children patients and ALL diagnose was confirmed.

Though, in both groups of patients with ALL and AML various number of AML1 extra copies was proved, in no one of them a high-level amplification was demonstrated. Gain of AML1 gene in all cases originated in polysomy of chromosome 21 (usually trisomy or tetrasomy) but one or more copies localized on marker chromosome were frequently found.

Mostly one copy of AML1 was included in one marker chromosome with extra material added of unknown origin (patient 2, 6, 15), just in patient 13 a duplication of long arm of chromosome 21 with tandem duplication of AML1 gene and another extra copy of AML1 in ring chromosome with unidentified material was shown. Similar cases of tandem repetition of 21(q11q22) with or without AML1 increase were described by others [10, 14, 17, 18, 19]. Alike intrachromosomal amplification by tandem repeat presence of AML1 copies in size-variable ring chromosomes have been reported in both types of leukemia even though predominately in myeloid malignancies in adults [11, 17, 19]. In contrast with this, our patient with ring chromosome was, exceptionally, 3-year-old girl with de novo AML M2.

ANDERSEN et al [5] classified as amplification of AML1 gene if, per metaphase cell, two and more signals were located on the same chromosome arm or two and more extra single signals of AML1. Extra copies of chromosome 21 with concordant gains of AML1 they did not consider.

Table 1. Data for childhood patients with extra copies of AML1 gene

<table>
<thead>
<tr>
<th>Patient No. (sex, age*)</th>
<th>Phenotype</th>
<th>Karyotype</th>
<th>Number of AML1 signals/cell</th>
<th>Other changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (M, 12) cALL</td>
<td>nk</td>
<td>+53,XY,+2,+4,+6,+17,+21,+2</td>
<td>3 – 5</td>
<td>-TEL, +6</td>
</tr>
<tr>
<td>2 (M, 9) cALL</td>
<td></td>
<td>±54,XY,+3,+5,+6,+7,+8,+1</td>
<td>4</td>
<td>+5,+6,+8</td>
</tr>
<tr>
<td>3 (M, 4) cALL</td>
<td>nk</td>
<td>+52,XXY,+5,+6,+17,+21,+21</td>
<td>4</td>
<td>+5,+6,+17,+X</td>
</tr>
<tr>
<td>4 (F, 4) cALL</td>
<td>nk</td>
<td>50-54,XX / 46,XX</td>
<td>4</td>
<td>/</td>
</tr>
<tr>
<td>5 (F, 5) cALL</td>
<td>nk</td>
<td>46,XX</td>
<td>4</td>
<td>/</td>
</tr>
<tr>
<td>6 (M, 4) cALL, cocexp. CD13, CD33</td>
<td>nk</td>
<td>+5, 2x +5, +6,+17,+X</td>
<td>/</td>
<td>/</td>
</tr>
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Table 2. Data for adult patients with extra copies of AML1 gene

<table>
<thead>
<tr>
<th>Patient No. (sex, age*)</th>
<th>Phenotype</th>
<th>Karyotype</th>
<th>Number of AML1 signals/cell</th>
<th>Other changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 (M, 55) AML</td>
<td>48,XY,+21,+m</td>
<td>3</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>15 (M, 74) MDS, AML</td>
<td>±90,XY,del(5q),+y1-15, +m1-7,+19, 48,XY</td>
<td>4–6</td>
<td>5q-, 2x +5p, +TEL, 4-8x c-MYC</td>
<td></td>
</tr>
<tr>
<td>16 (F,42) AML M2</td>
<td>49,XY,+4,+8, +21 (3) / 46,XX</td>
<td>3</td>
<td>+8</td>
<td></td>
</tr>
<tr>
<td>17 (F,55) AML M1</td>
<td>47,XX,del(11)(q23),+21 / 46,XX</td>
<td>3</td>
<td>MLLs-</td>
<td></td>
</tr>
<tr>
<td>18 (F,30) pre–B ALL</td>
<td>46,XX</td>
<td>6</td>
<td>TEL/AML1, TEL/AML1, -TEL, -TEL</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Interphase nuclei FISH of patient 18. Using dual color translocation TEL/AML1 probe uncovered two yellow fused signals for TEL/AML1, 4 extra orange signals for AML1 gene and loss of normal (green) TEL allele.
tected in other 6 patients without fusion. Neither JABBAR AL-OBAIDY nor PENTHER et al [4] registered increase of AML1 signals more than 4. With a dual-color FISH probe for AML1 and TEL two fusion signals and another 4 orange signals for AML1 were seen in interphase nuclei of our patient. With normal karyotyp 46,XX and without FISH on metaphase was impossible to define type and localization of amplified AML1 gene.

The resembling gain of AML1 was observed also in patient 15, with clone of near tetraploid metaphases and complex karyotypes. Besides the 4–6 copies localized in several markers, FISH revealed C-MYC amplification cytogenetically presented like double minute chromosomes (dmin). HILGENFELD et al [22] probed recurring involvement of chromosome 21 and amplification of the MYC oncogene in AML M2. Albeit dmin represents an infrequent phenomenon in AML and they are found in only about 1% of cases, in their study of 140 patients two of them displayed C-MYC amplification together with trisomy of AML1 gene. Anyway, our patient 15 was not an extraordinary event, however association between gains of both oncogenes stay unclear yet.

Another specific finding in our study was presented in patient 12, a 4-year-old girl with common ALL phenotype and primary trisomy of chromosome 21; FISH analyses confirmed AML1 extra copy as well as uncovered partial deletion of long arm of chromosome 7. This combination was already observed [23], however, in connection with myeloid malignancies.

In conclusion, AML1 gene amplification can occur through polysomy of chromosome 21 or through true high-level amplification due to repetition in tandem fashion and/or presence of AML1 copies on extrachromosomes. We have confirmed that increase of AML1 most frequently originated from trisomy or tetrasomy of chromosome 21 in both ALL and AML. HARRWOOD et al [17] suggested that cases of high-level amplification should be distinguished from cases of polysomy of chromosome 21. In case of polysomy, implication of other oncogenes located on chromosome 21 can not be excluded. In case of true high-level amplification, AML1 seems to be only amplified gene. We have not proved higher incidence of high-level amplification but in other side we have observed some noteworthy and uncommon changes of chromosome 21 with correlation of AML1 gain and either aberrations or age and diagnoses, respectively. Our observations might contribute to define the exact incidence of AML1 amplifications and their correlation with specific clinical characteristics.

The authors wish to thank Dr. SEJNOVÁ (University Children Hospital, Bratislava, SR), Dr. BUŠASKA (Roosevelt Hospital, Banska Bystrica, SR), Dr. JENČO (University Hospital, Košice, SR), Dr. DEMITROVIČOVÁ (NCI, Bratislava, SR) and Dr. KOTOUBEK (University Hospital, Bratislava, SR) for providing the clinical data.

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