

1. Introduction

The t(12;21)(p13;q22) translocation is the most common genetic alteration in childhood B-cell acute lymphoblastic leukemia occurring in approximately 25% of cases and is associated with a favorable outcome [1, 2].

The molecular consequence of this translocation is the fusion of two known genes: ETV6 mapped to 12p13 and RUNX1 located at 21q22 [1, 3]. It results in the formation of a hybrid protein involving the helix-loop-helix domain of ETV6 and the entire RUNX1 gene that appears to interfere with transactivation by the normal RUNX1 in a transdominant manner [3, 4].

Because it is a cryptic translocation which usually escapes diagnosis on conventional cytogenetic (CC) study due to the similarity of the changed bands, molecular cytogenetic tools such as fluorescence in situ hybridization (FISH) are needed to determine the incidence of the ETV6-RUNX1 fusion in the Tunisian B-lineage ALL pediatric cases that had normal karyotypes or random chromosome aberrations at diagnosis and to compare our findings to those previously described in literature.

2. Methods

2.1. Patients. Among 57 childhood B-lineage ALL cases analyzed by conventional karyotype, 41 were selected for FISH analysis according the following criteria: age (0–16); absence of cytogenetically detectable recurrent abnormalities, that is, high hyperdiploid karyotypes (51–65 chromosomes), t(4;11), t(1;19), and t(9;22). Theses patients are referred to our laboratory for cytogenetic analyses from different hematological services in Tunisia.

2.2. Conventional Cytogenetics. Chromosomal preparations with RHG-banding were performed according to a previously described protocol [5].
Chromosomes were identified and arranged according to the international system for human cytogenetic nomenclature (ISCN 2005) [6], and the karyotype profile was determined by the analysis of at least 20 metaphases.

2.3. Fluorescence In Situ Hybridization (FISH). The presence of the ETV6-RUNX1 fusion gene was assessed using two YAC clones (the YAC936E2 and the YAC821S11) selected from their location in the 12p13 (ETV6) and the 21q22 (RUNX1) regions, respectively, in the “Centre d’Etude du Polymorphisme Humain Database” (http://www.cephb.fr/).

The ETV6 and the RUNX1 probes were labeled with tetramethylrhodamine-6-dUTP and fluorescein 12-dUTP (Abbott-Vysis, Downers Grove, IL, USA), respectively, using nick translation kit (Abbott-Vysis, Downers Grove, IL, USA).

For FISH technique, slides were denatured in 70% formamide at 75 °C for 1 minute and 30 seconds then dehydrated with cold (−20 °C) 70%, 85%, and 100% ethanol for 1 minute and 30 seconds each. After drying, the ETV6-RUNX1 dual-color FISH probe, denatured as preliminary at 75 °C for 5–10 minutes, was applied into a slide, covered with a 24 × 24 mm coverslip and sealed with a rubber cement. Slides were then placed in a humid light-proof container at 37 °C for overnight hybridization.

After hybridization, the coverslip was removed and slides were washed in 0.4 standard saline citrate (SSC) for 5 minutes at 75 °C, followed by a second wash in 2XSSC-0.1% NP40 for 2 minutes.

After drying, the slides were counterstained with 4',6-diamidino-2-phenylindole and examined with a fluorescent microscope equipped with appropriate filters and CytoVision FISH system image capture software (Zeiss Axioskop 2 plus) and at least 50 metaphase cells, and 100 interphase nuclei were analyzed for ETV6-RUNX1 translocation.

In a normal case, the hybridization with the ETV6 (red) and the RUNX1 (green) probes showed two red and two green signal patterns (Figure 1(a)). However, in a case with the t(12;21), the hybridization with these probes showed one or two fusion signals (red/green or yellow), one red and one green signal patterns corresponding to the normal copies of the ETV6 and the RUNX1 genes, respectively, (Figure 1).

3. Results

Among 57 children with B-lineage ALL, 16 cases were not screened for this study (4 cases with t(9;22)(q34;q22) from which one was also associated to a hyperdiploid karyotype with 53 chromosomes, 1 case with t(1;19)(q23;p13), 1 case with t(8;14)(q24;q32), 2 cases with t(4;11)(q21;q23), 2 cases with del(9)(p12p23), 1 case with del(11)(q23), 3 cases with del(6q), and 2 cases with high hyperdiploidy (53 and 56 chromosomes). Both are associated to an add(14)(q32).

The selected 41 cases included 25 males and 16 females with age ranging from 1.2 to 15 years (mean 7.4 years).

Twenty-three out of the 41 patients (56%) had abnormal FISH findings (Table 1).
Table 1: Summary of patients with abnormal FISH findings.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age/sex (years)</th>
<th>Karyotype (number of cells)</th>
<th>FISH signals (No.)</th>
<th>% of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ETV6 RUNX1 ETV6-RUNX1 fusion</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>13/M</td>
<td>46, XY[20]</td>
<td>0 1 2</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>2/M</td>
<td>46, XY[16]</td>
<td>0 1 1</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>5/M</td>
<td>46, XY, del(20)(p12)[8]/46, XY[10]</td>
<td>1 1 2</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>6/M</td>
<td>46, XY[19]</td>
<td>1 2 1</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>3/F</td>
<td>46, X, −X, add(3)(q27), + mar[5]/46, XX[15]</td>
<td>3 3 0</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>15/F</td>
<td>48, XX, t(2;11)(p12q23), +20, +21[18]</td>
<td>2 3 0</td>
<td>24</td>
</tr>
<tr>
<td>7</td>
<td>13/M</td>
<td>46, XY, −16, +mar[9]/46, XX[6]</td>
<td>2 3/4 0</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>2/F</td>
<td>46, XX[20]</td>
<td>2 4 0</td>
<td>19</td>
</tr>
<tr>
<td>9</td>
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<td>14</td>
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<td>32</td>
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<td>17</td>
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<td>15</td>
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<td>1 1 1</td>
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<tr>
<td>16</td>
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<td>2 3 0</td>
<td>14</td>
</tr>
<tr>
<td>17</td>
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<td>47, XXY, del(12)(p12−p13)[8]/47, XXY[12]</td>
<td>2 3 0</td>
<td>16</td>
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<tr>
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<td>47, XX, +5[9]/46, XX[3]</td>
<td>1 1 1</td>
<td>17</td>
</tr>
<tr>
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<td>1 1 1</td>
<td>22</td>
</tr>
<tr>
<td>23</td>
<td>1.2/M</td>
<td>46, XY[19]</td>
<td>2 3 0</td>
<td>14</td>
</tr>
</tbody>
</table>

*This case harbored two distinct clones: one with only the ETV6/RUNX1 rearrangement and the second (9% of cells) with an extra signal of RUNX1 gene.

No: number.

4. Discussion

In the present study, we used FISH for the detection of ETV6-RUNX1 fusion gene in 41 children with B-lineage ALL that had normal karyotypes or random chromosome aberrations at diagnosis. To our knowledge, this is the first report of the frequency of the t(12;21) from Tunisia and the second from an Arab country after the Egyptian study published by Mikhail et al. in 2002 [7].

ETV6-RUNX1 rearrangement was present in 16 of the 57 selected subgroup of Tunisian patients with B-lineage ALL (28%). Of them, 13 (13/16, 81%) had a normal karyotype at diagnosis, a percentage higher than previously reported by Veiga et al. in Brazil (6/12, 50%) [8] and by Douet-Guilbert et al. [9] in France (2/10, 20%).

We confirm the efficiency of FISH technique in the detection of such cryptic chromosomal rearrangement, usually missed by CM [10, 11].

In Egypt, UK, India, Korea, and Malaysia, ETV6-RUNX1 rearrangement was found with a lower frequency than we have found, ranging from 0 to 22% [7, 12–14].

However, in B-lineage ALL patients of Germany [11], Italy [11], and France [15], the frequency of this translocation is reported to be about 25%. A frequency of 31% is...
found among American patients [16], and we recently report in Israel [17] and in Brazil [8] an incidence of 40%.

This difference could be explained by the fact that due to the expected peak incidence at 3–5 years reported in the majority of the published series, our cases could have been biased for old age (mean age 7.4 years), which then corresponds to lower t(12;21) incidence.

The ETV6-RUNX1 fusion gene on the der(21) was detected in all patients with t(12;21), whereas the reciprocal ETV6-RUNX1 fusion gene on the der(12) was observed in only two cases.

While this t(12;21) may initiate the leukemic process, critical secondary genetic events found in association with ETV6-RUNX1 fusion gene are currently believed to be pivotal for leukemogenesis and are previously reported in literature [4, 18–20].

In our study, additional chromosomal aberrations were found in five patients among ETV6-RUNX1 fusion positive cases (31.2%).

Two patients (12.5%) showed deletion of the nontranslocated ETV6 allele. This deletion has been reported in several studies but its incidence is highly variable, ranging from 8.6% to 87.5% [2, 7, 9, 21–23].

This low detection of del(12) in our study could be explained by the fact that the ETV6 FISH probe covered almost the entire of the ETV6 gene so that submicroscopic deletion could be missed.

According to the Knudson’ hypothesis [9], ETV6 could act as a tumor suppressor gene, with t(12;21) disrupting one copy of ETV6 and the deletion being the second inactivating event [17, 21].

In the three other patients, we have found extra copies of the RUNX1 gene in which the clone with the extra signal of RUNX1 was distinct from the one harboring ETV6-RUNX1 fusion gene.

Interestingly, extra copies of RUNX1 gene were also observed in 7 patients without ETV6-RUNX1 fusion. Of them, three do not have +21 on their karyotype which is likely consistent with extra 21 perhaps in a small clone not observed cytogenetically or derive from non dividing clonal hyperdiploid cell, while normal metaphases derived from normal dividing cells.

In the four remaining cases, the number of RUNX1 signals was in keeping with results on CC including a case (case 5) in which we have identified a t(3;12) by FISH. This translocation has previously been reported in a case with B-lineage ALL but in association with t(12;21) by Yehuda-Gafni et al. [17] and Kobayashi et al. [19].

Extra copies of the RUNX1 gene were found in 6 patients (having or not ETV6-RUNX1 fusion gene and without +21 in the karyotype) among 57 patients with B-lineage ALL (10.5%). This incidence was higher than that previously reported by Dal Cin et al. [24], Niini et al. [25] and more recently by Busson Le-Coniat et al. [26] who reported only 2 or 3 cases.

Although the real significance of these findings has not been clarified yet, the hypothesis of the gene-dosage effect involving RUNX1 seems to be very probable [27, 28].

In summary, our findings demonstrate that ETV6-RUNX1 fusion gene is a common genetic abnormality detected by FISH in 28% of Tunisian children with B-lineage ALL, and we confirm that secondary genetic events are commonly encountered in these patients. Interestingly, extra copies of RUNX1 are frequently found in our series than those previously reported in other populations and so warrant further investigation to elucidate the mechanisms underlying the role of the RUNX1 in leukemogenesis. Moreover, due to the unknown prognostic significance of this abnormality, further studies should be conducted in consecutive children with ALL to correlate RUNX1 overexpression with the patients’ followup.

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References


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