Trisomy 13 correlates with RUNX1 mutation and increased FLT3 expression in AML-M0 patients

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ABSTRACT

Of 52 AML-M0 patients studied, 16 presented a RUNX1 mutation (30.8 %) and 8 carried a trisomy 13 (15 %). We found a strong correlation between trisomy 13 and RUNX1 mutations, i.e., 7 out of 8 cases with trisomy 13 carried a mutation in RUNX1 (87.5 %, p<0.00056). Trisomy 13 patients with a RUNX1 mutation showed a 4-fold higher expression of FLT3 mRNA compared to controls, and in a selected number of cases, a higher cell fraction expressing FLT3 and an increase in the number of FLT3 receptors at the cell surface. In conclusion, our results show that trisomy 13 is correlated to RUNX1 mutation and increased FLT3 expression in AML-M0.

Key words: acute myeloid leukemia, trisomy 13, RUNX1, FLT3, AML-M0.

Material and Methods

Patients material

Patient material, morphologically and immunophenotypically classified as AML-M0, was kindly provided by JC Kluin-Nelemans, University of Groningen, The Netherlands, B. Löwenberg, Erasmus University Medical Center, Rotterdam, The Netherlands, W-D Ludwig, Medical University of Berlin, Germany, WAF Marijt, Leiden University Medical Center, The Netherlands and WR Sperr, Medical University of Vienna, Austria. Pure tumor populations were obtained by sorting mononuclear cells using flow cytometry as previously described. DNA and RNA were isolated using commercially available kits (Qiagen, Hilden, Germany).
**Mutation screening**

*RUNX1* mutation screening was performed as previously described.\(^{13}\) Conditions and primer sequences for *FLT3* ITD and D835 mutation screening are described in the online Supplementary Appendix.

**FLT3 expression**

Primer sequences and details of the conditions used for Quantitative real-time PCR (qPCR) are described in the online Supplementary Appendix. Amounts were normalized using the geometrical mean of the housekeeping genes *GAPDH*, *HPRT* and *YWHAZ*. The control panels consisting of cDNA from AML-M0 patients are described in Figure 1. For flow cytometric analysis monoclonal antibodies anti-CD34 (Becton Dickinson, San José, CA, USA), anti-CD117 (Dako, Glostrup, Denmark), anti-CD135 (Immunotech, Marseille, France) and anti-CD45 (Becton Dickinson) were used. Antibodies were FITC, APC, PE and PERCP conjugated respectively. The antibodies were added to 5x10^6 mononuclear cells and incubated for 30 minutes in 100 μL PBS containing 0.1% BSA. After washing, the cells were resuspended in 500 μL PBS containing 0.1% BSA. Flow cytometry analysis was performed using a FACSCalibur (Becton Dickinson).

**Results and Discussion**

We screened 52 AML-M0 patients for mutations in *RUNX1* (exons 3, 4 and 5), *FLT3* (ITD and D835), *N* and *KRAS*, *PTPN11* and *JAK2* (manuscript in preparation). We found *RUNX1* mutations in 16 patients (30.8%) and *FLT3*, *RAS*, *PTPN11* or *JAK2* mutations in 22 patients of the cohort. *FLT3* ITD mutations were detected in 2 out of the 16 cases with *RUNX1* mutation. Of the 52 patients, material from 8 patients showed extra copies of chromosome 13 (15%, Table 1), a high percentage which agrees with published reports.\(^{1,7}\) Seven out of the 8 patients with trisomy 13 (87.5%) showed mutations in *RUNX1* (Table 1), while 7 out of the 16 patients with *RUNX1* mutations (44%) had trisomy 13. Statistical analysis (one-sided Fisher’s exact test) showed that trisomy 13 and *RUNX1* mutation co-occurred more frequently than expected by chance (p<0.00056). Conversely none of the trisomy 13 AML patients showed mutations in *FLT3*, *RAS*, *PTPN11* or *JAK2*, with the exception of patient 30 who had a mutation in *PTPN11*. A negative correlation between *FLT3* mutation and trisomy 13 has been previously reported.\(^{14}\) Our results are supported by a substantial co-occurrence of *RUNX1* mutation and trisomy...
Trisomy 13 found in literature, as of 26 samples with RUNX1 mutation and a known karyotype, 5 also had trisomy 13 (19.2%). It has been hypothesized that two classes of mutations, differentiation and proliferation, are necessary and sufficient to lead to leukemogenesis. Therefore, given the known role of FLT3 in AML, FLT3, located on chromosome 13, is a very likely candidate for deregulation as a result of trisomy 13.

FLT3 mRNA expression was studied in 6 out of 8 patients (Table 1). Trisomy of chromosome 13 was examined by quantitative PCR. FLT3 expression was significantly and consistently 3-fold higher in the trisomic samples compared to a mixed panel of AML-M0 controls (Figure 1A). To test how specifically FLT3 expression was affected, the neighboring CDX2, PAN3 and FLT1 genes and FOXO1A (also on chromosome 13) were used as controls. FOXO1A has been reported to be over-expressed in AML while CDX2, PAN5 and FLT1 have been found amplified in a small chromosome 13 region in three AML patients. FOXO1A and PAN3 expression was not increased in the trisomic samples compared with the controls (Figure 1A). FLT1 expression was not detected in any of the cases and CDX2 expression was restricted to a fraction of the patients without correlation to trisomy 13 (data not shown). These results argue in favor of a specific deregulation of FLT3 beyond dose effect.

This study has shown trisomy 13 to be strongly correlated to RUNX1 mutation. Therefore, we also evaluated whether RUNX1 mutation was itself sufficient for increased FLT3 expression. AML-M0 patients with a RUNX1 mutation without trisomy 13 have a consistently 2-fold higher FLT3 expression compared to the AML-M0 controls (Figure 1B). Cases with both trisomy 13 and RUNX1 mutation show a statistically significant 4-fold increase in FLT3 expression (Figure 1B). The increased fold change associated with RUNX1 mutation alone also explains the difference between the fold changes detected in the trisomy 13 samples (3- versus 4-fold, Figure 1A versus Figure 1B) as patients with RUNX1 mutations where included in the control panel of the first experiment. Clearly, neither RUNX1 loss nor trisomy 13 alone can account for a 4-fold increase in FLT3 expression in these patients. Although there could be several explanations, it is possible that trisomy 13 and RUNX1 loss have a synergistic effect on FLT3 expression in these cases. This could also account for the specificity of FLT3 up-regulation when compared to FOXO1A and PAN3.

In three cases with FLT3 ITD and one with FLT3 D835 but without trisomy 13, expression of FLT3 was not increased, adding to the conflicting data available on the increase in FLT3 expression in FLT3 mutated cases (Figure 1B).

We were able to study FLT3 protein expression in two patients (27 and 31) with trisomy 13 using FACS. The analysis was restricted to the CD34 expressing cell population. As FLT3 and KIT are co-expressed in normal CD34+ bone marrow cells we also studied KIT expression. The cell fraction expressing FLT3 is much higher in patients 27 and 31 than in any of the AML-M0 controls (p<0.0026, t-test, Figure 1C). Also, the FLT3/KIT fraction in these two patients was higher (p<0.0036, t-test) and seemed to negatively correlate with the FLT3- and KIT+ population. Finally, we studied the median fluorescence intensity of FLT3 to measure the number of receptors at the cell surface. In patients carrying a trisomy 15 (27 and 31) the median fluorescence intensity of the FLT3 positive population was considerably higher than in the controls (p<0.0086, t-test, Figure 1D). Correlation between FLT3 mRNA expression and protein expression at cell surface has been previously reported, although there are conflicting data. A role for high FLT3 expression in AML leukemogenesis has been hypothesized. In some cases, over-expression of FLT3, at levels comparable with this study, was shown to result in auto-activation of this receptor. From a mechanistic point of view, it is interesting to speculate whether over-expression of normal FLT3 receptor, activated either by ligand or by auto-phosphorylation, is comparable to FLT3 mutations. Within this context, trisomy 13 would be an alternative to FLT3 ITD and D835 mutations. This hypothesis would explain the lower frequency of overlapping FLT3 and RUNX1 mutations.

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**Table 1. Karyotype and RUNX1 mutation status.**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Karyotype</th>
<th>RUNX1 mutation</th>
<th>FLT3 ITD and D835 mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>7♂</td>
<td>47,XY,+13,(17)(q10)</td>
<td>W79C (H)</td>
<td>wt</td>
</tr>
<tr>
<td>27♂</td>
<td>47,XY,+13</td>
<td>R80H (H)</td>
<td>wt</td>
</tr>
<tr>
<td>30♀</td>
<td>47,XY,+13</td>
<td>W79C (H)</td>
<td>wt</td>
</tr>
<tr>
<td>31♀</td>
<td>47,XY,+13</td>
<td>W79C (H)</td>
<td>wt</td>
</tr>
<tr>
<td>32♀</td>
<td>47,XY,+13</td>
<td>D171V (H)</td>
<td>wt</td>
</tr>
<tr>
<td>36♂</td>
<td>47,XY,+13</td>
<td>R142h (H)</td>
<td>wt</td>
</tr>
<tr>
<td>46♀</td>
<td>47,XY,+13</td>
<td>Not detected</td>
<td>wt</td>
</tr>
<tr>
<td>53♀</td>
<td>46,XY,der(13)(q13;24q22),+19,+19</td>
<td>Total Deletion (H)</td>
<td>wt</td>
</tr>
</tbody>
</table>

*All trisomies confirmed by SNP analysis. a) used for FLT3 Real Time PCR quantification; b) +13 detected with SNP array; c) detected with SNP arrays. H: homozygous; h: heterozygous; wt: wild type.*
mutations found by us compared to another study, as this study has a lower incidence of trisomy 13 than expected. A high frequency and correlation of trisomy 13 and RUNX1 mutations has been recently reported in an abstract including AML subtypes beyond the M0, suggesting that this might be a general mechanism for leukemia in AML.

In conclusion, we have shown that trisomy 13 is highly correlated with RUNX1 mutation and that FLT3 mRNA expression is greatly increased in tumor cells from AML-M0 patients where both abnormalities occur. The high FLT3 mRNA expression translates into clear increase in both the cell fraction expressing FLT3 and the number of FLT3 receptors at the cell surface in at least two patients. Given the function of FLT3, its involvement in AML and the distribution with other mutations, our data suggest that up regulation of FLT3 may play an important role in AML-M0 with trisomy 13 and RUNX1 mutation.

References

17. Tefferi A, Pardanani A Mutation screening for JAK2V617F: when to order the test and how to interpret the results. Leuk Res 2006;30:739-44.