t(10;12) (q24; p13) as the sole abnormality in a case with refractory acute myeloid leukemia: The first case report and literature review

Gary LU ∆, Inga GUREVICH, Binh T VO, Su S. CHEN
(Department of Hematopathology, University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA)

SUMMARY Rearrangements involving chromosome region at 12p13 are common abnormalities in hematological malignancies, including myeloid and lymphoid types. ETV6 gene is usually involved in the 12p13 region. ETV6 rearrangements are more often observed in acute lymphoblastic leukemia than in acute myeloid leukemia (AML), where ETV6 gene deletions are more common than rearrangements. Here, we report an AML case with the recurrent t(10;12) (q24; p13) as the sole abnormality. Fluorescence in situ hybridization with mapping back to metaphases confirmed that the ETV6 gene splits, and rearranges with a locus at 10q24. In review of the literature, this is the first report of AML case with the novel abnormality as the sole change. Complete laboratory findings from bone marrow examination, flow cytometry analysis, cytogenetic studies, molecular analysis, and clinical features are also described in the report.

KEY WORDS Leukemia, myeloid, acute; Gene rearrangement; t(10;12); (q24; p13)

Clonal chromosomal abnormalities play an important role in classification, diagnosis, treatment, and prognosis of hematological malignancies1-4. For example, the t(9;22) (q34;q11.2) resulting in a BCR/ABL1 fusion gene is most commonly associated with chronic myelogenous leukemia (CML) whereas the t (15;17) (q22;q12) leading to a PML/RARA product is specific for acute promyelocytic leukemia (APL)1-3. Rearrangement of ETV6 gene mapped to 12p13 region is heterogenotypic in that the change can be associated with either myeloid disorders or lymphoid malignancies depending on the chromosome translocation partner involved. However, unlike the t(12;21) (p13;q22), which is specifically associated with pediatric acute lymphoblastic leukemia in about 25% of cases4,5, chromosomal translocations involving 12p13/ETV6 in myeloid disorders are usually not specific for certain subtype of acute myeloid leukemia (AML), and the translocated partners are diverse. So far, at least 24 different partners for the 12p13/ETV6 have been reported; the number of the myeloid cases showing each of the known partner is low, usually below a dozen5. Therefore, identification of more AML cases with translocation involving 12p13/ETV6 is appreciated. We are reporting a t(10;12) (q24; p13) as the sole abnormality in a patient with refractory AML evolving from myelodysplastic syndrome. To our knowledge, this is the first AML case with the t(10;12) as the sole abnormality.

1 Patients and Methods
A 47-year-old male was diagnosed with myelodysplastic syndrome (MDS) when he first presented to the clinic with fatigue, itchy feet and then bruises all over the body in December 2006. The patient was referred to MD Anderson Cancer Center in February 2007. Results of bone marrow aspirate examination, demonstrating 11% blasts, supported a diagnosis of high-risk myelodysplastic syndrome. Cytogenetics study at that time revealed a normal diploid karyotype. The patient was then treated with four cycles of decitabine in addition to red cell and platelet transfusions due to his pancytopenia. However, he did not respond to the chemotherapy, and underwent a protocol for Busulfan-Fludarabine-Clofarabine with allogeneic stem cell transplantation (ASCT) 9 months after his initial diagnosis of MDS, but relapsed 116 days post ASCT. Bone marrow examination at that time demonstrated 54% blasts. The patient refused further treatment due to rapid progression of the disease and his worsening health condition, and deceased 25 months after his initial diagnosis of MDS. According to the findings and based on WHO definition1, a diagnosis of acute myeloid leukemia evolving from myelodysplastic syndrome was made.

2 Results
2.1 Bone marrow morphology examination
Wright-Giemsa-stained peripheral blood and bone marrow aspirate smears and hematoyxin-eosin-stained sections of bone marrow aspirate clot and core biopsy specimens were reviewed. Cytological stains for myeloperoxidase and butyrate esterase were performed on aspirate smears with increased blasts using conventional methods. The diagnosis of AML was based on the World Health Organization classification of hematopoietic tumors6.

Blast count in consecutive bone marrow aspirate samples before ASCT remained elevated, ranging from 2% to 10% during the period of MDS. Bone marrow features during relapse included marked increase in blasts ranging from 54% to 74% with morphology showing intermediate to large cells, fine nuclear chromatin, one or two nucleoli and abundant sparsely granular cytoplasm. A subset of blasts contained fine cytoplasmic vacuoles and showed monocytic/monoblastic features (Figure 1). No Auer rods were identified, and the bone marrow demonstrated extensive fibrosis.

Figure 1 Bone marrow aspirate shows a packed marrow with mostly immature myeloid and monocytoid cells.
(Wright-Giemsa, 50 x magnification)

△ Corresponding author’s e-mail, gglu@mdanderson.org
2.2 Flow cytometric analysis

At the initial diagnosis, four-color flow cytometric analysis at MD Anderson Cancer Center with monoclonal antibodies included CD3, CD4, CD8, CD10, CD13, CD16, CD19, CD34, CD36, CD56, CD71, kappa and lambda [7]. All antibodies were from BD Biosciences (San Jose, CA). Analysis was performed using FACS Caliber cytometers (BD), demonstrating expression of CD13, CD16 and CD10. The immunophenotypic abnor-

minalities were suggestive of myelodysplastic syndrome. At the time when the patient was in relapse, flow cytometric analysis with acute leukemia panel revealed positivity for CD13, HLA-DR, CD33, CD14, CD117, CD34, and MPO, supporting the diagnosis of AML and consistent with mononuclear differentiation.

2.3 Cytogenetics and fluorescence in situ hybridization

Conventional G-banded karyotyping was performed on cul-
tured metaphase cells from bone marrow aspirate specimens. The samples were cultured for 24 to 48 hours using the protocol for routine clinical cancer cytogenetics laboratory at MD Anderson Cancer Center. The cultures were harvested with Metaphase Harvester HANABI-II (Chiba, Japan). After hypotonic treat-

ment and fixation with 3:1 methanol acetic acid solution, cell suspensions were dropped on clean slides using THERMOTRON (Thermotron Industrial Inc., MI, USA). G-bandning was performed after the slides were dried at 60 °C overnight. A normal diploid 46, XY was revealed at the initial diagnosis of MDS whereas a clonal t(10;12) (q24;p13) was detected in all the 20 cells analyzed (Figure 2) when the patient was in relapse.

Commercially available LSI ES ET6V probe (Abbott Molecular, Inc, Illinois, USA) was used for fluorescence in situ hybridization (FISH) analyses. The probe is useful to detect ET6V gene rearrangement mapped to 12p13 region. For interphase FISH, stan-

dard protocol used in the Cytogenetic Laboratory at MD Anderson Cancer Center was employed [9]. For FISH mapping-bank to G-banded metaphase cells, metaphases to be exam-

ined were located and marked according to the coordinates for routine cytogenetics analysis. Slides were distained in fixative solution (3:1) for 2 minutes, incubated in 2 × SSC solution at 37 °C for 30 minutes, and dehydrated in a series of ethanol (70%, 85%, 100%) for 2 minutes each, and dried at room temperature. 10 microliter of probe mixture was applied on the target area with a 22 mm × 22 mm coverslip, and then sealed with rubber cement. The slide and probe were co-denatured at 72 °C for 2 minutes using HybridII™ instrument (Abbott Molecular, Inc.). Hybridization was done in incubator at 37 °C overnight. After hybridization, the slides were washed according to the manufacturer’s procedure. The slides were ready to be an-

alyzed after DAPI/antifade application.

2.4 Molecular studies

Molecular studies included analyses for presence of muta-

tions in JAK2 codon 617, KRAS and NRAS codons 12, 13, and 61, FLT3 internal tandem duplication and codon 835/836, and NPM1 exon 12. DNA was extracted from peripheral blood or bone marrow aspirates using Gentra AUTOPURE LSTM automated DNA extraction system (Qiagen Systems, Valencia, CA) and was amplified by PCR using gene specific primers. PCR products were then either sequenced by Pyrosequencing PSQ96 HS System (Biotage AB, Uppsala, Sweden) for JAK2, KRAS, and NRAS mutation analysis, or subjected to DNA fragment size analysis using 3130 Genetic Analyzer (Applied Biosystems Inc., USA) for FLT3 and NPM1 mutation analysis. None of these mutations was detected (data not shown).

3 Discussion

Anomaly of ET6V gene in acute myeloid leukemia is often due to loss of function, resulting from chromosomal aberrations such as deletion or other aberrations involving 12p13 region, loss of chromosome 12, or ET6V gene mutations. Thus, the ET6V gene is consi-

dered a tumor suppressor gene. However, the leukemogenesis mechanism of ET6V rearrangement resulted from chromosomal translocations involving 12p13 region can be vari-

able, most commonly resulting in ET6V over-expression due to the formation of a fusion gene, similar to the cases of acute pro-

myelocytic leukemia (APL). In APL, the retinoic acid receptor alpha (RARA) gene on 17q21 fuses with a nuclear regulatory factor gene PML on 15q22 resulting in a hybrid gene 5′PML-3′

RARA due to the specific t(15;17) (q22;q21). The expression of the PML/RARA fusion gene block leukemic cell differentiation and induce cell-cycle arrest [10-12]. ET6V gene rearrangement is common in acute lymphoblastic leukemia (ALL), particularly pediatric ALL, accounting for at least 25% of pediatric ALL; whereas the non-rearrangement is more common in AML cases [12]. Partners for the ET6V rearrangement are diverse. So far, there have been at least different 24 loci translocated with the ET6V gene in acute myeloid leukemia [5]. Identification of new chromosomal partner with the 12p13 would be of clinically signifi-

G-banded karyotype showing t(10;12) (q24;p13) analyzed with Applied Imaging software (Santa Clara, CA). At relapse, ET6V rearrangement was positive in 164 of 200 (82%) interphase cells analyzed (Figure 3A). FISH mapping-back to G-banded metaphases confirmed a t(10;12) (q24;p13) (Figure 3B).


FISH analysis was performed by using a suitable filter set on an optimally performing fluorescent microscope. Each fluoro-


toplane-labeled probe was viewed by an optical filter according to the manufacturer’s instructions. All FISH images were captured and


Figure 2 G-banded karyotype showing t(10;12) (q24;p13)

Figure 3 Interphase-FISH pattern (A); FISH in mapping-back to G-banded metaphase (B)
In review of the literature, the recurrent t(10;12) (q24; p13) has been reported in four cases of myeloid malignancies (Table 1) [13-16].

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex/age (years)</th>
<th>Diagnosis</th>
<th>Previous disease</th>
<th>Karyotype</th>
<th>Morphology feature</th>
<th>ETV6 rearrangement involved</th>
<th>Gene involved at 10q24</th>
<th>Therapy response/survival</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/77</td>
<td>CMML</td>
<td>Refractory anemia</td>
<td>46, XY, t(10;12) (q24; p13) [NA]</td>
<td>Monocytosis, eosinophilia</td>
<td>Yes</td>
<td>Unknown</td>
<td>NA/20 months*</td>
<td>Wlodarska et al [13]</td>
</tr>
<tr>
<td>2</td>
<td>n/a</td>
<td>CML blast crisis</td>
<td>CML</td>
<td>46, XX, t(9;22) (q34; p11.2), t (10;12) (q24; p13) [13]</td>
<td>NA</td>
<td>No</td>
<td>Unknown</td>
<td>NA</td>
<td>Aguiar et al [14]</td>
</tr>
<tr>
<td>3</td>
<td>M/77</td>
<td>RAEB</td>
<td>MDS</td>
<td>46, XY, t(10;12) (q24; p13) [in most cells]</td>
<td>Monocytosis, eosinophilia</td>
<td>Yes</td>
<td>GOTI</td>
<td>Refractory/NA</td>
<td>Janssen et al [15]</td>
</tr>
<tr>
<td>5</td>
<td>M/47</td>
<td>AML</td>
<td>Refractory MDS</td>
<td>46, XY, t (10;12) (q24; p13) [16]</td>
<td>Monoblastic</td>
<td>Yes</td>
<td>NA</td>
<td>Refractory/Deceased in 25 months</td>
<td>Present case</td>
</tr>
</tbody>
</table>

The patient still survived by the time the case was reported; AML, acute myeloid leukemia; CML, chronic myelogenous leukemia; CMML, chronic myelomonocytic leukemia; F, female; M, male; MDS, myelodysplastic syndrome; n/a, NA, not available; RAEB, refractory anemia with excess blasts.

However, only 3 of the 4 cases demonstrated the ETV6 rearrangement (cases 1, 3 and 4 in Table 1). No ETV6 gene rearrangement was detected in the case reported by Aguiar et al. [14]. Of the 3 cases with the t(10;12) involving ETV6 gene rearrangement, 2 de-monstrated an ETV6/GOTI fusion gene. Molecular studies revealed different ETV6/GOTI chimera between the two cases [15-16]. Mechanism of leukemogenesis of the ETV6/GOTI product is unclear. Inactivation of the ETV6 gene may be the consequences. Cytogenetically, the t(10;12) was observed as the sole abnormality during the course of the disease in the case reported by Janssen et al. [15]; whereas the abnormality is a second-dary change during the MDS course and when disease was transformed to AML in the other case by Struzki et al. [16]. With clinical data available, it appears that the features of myeloid malignancies with the t(10;12) (q24; p13) are monocytic morphology (cases 1, 3 and 5), refractoriness to chemotherapy (cases 3, 4 and 5), and unfavorable prognosis. Patient in case 4 by Struzki et al survived only 5 days, and the patient in our case deceased 25 months after diagnosis, in spite of intensive chemotherapy and stem cell transplantation. Data of partner gene of the ETV6 resulted from the t(10;12) in our case is not available; however, it appears that GOTI is also involved compared to the other two cases (case 3, 4). Molecular study is under investigation for our case to confirm the suspicion.

In conclusion, t(10;12) (q24; p13) is a recurrent cytogenetic abnormality associated with myeloid malignancies, monocytic morphology, and unfavorable prognosis. Our case described in this report is the first case of acute myeloid leukemia with the t(10;12) (q24; p13) as the sole abnormality. Genetic mechanism of tumorigenesis of the t(10;12) in AML needs to be further investigated.

References

[9] Lu G, Yin C, Medeiros J, et al. 15q deletion as sole abnor-
仅由 t(10;12) (q24;p13) 异常所致的难治性急性髓细胞白血病首例报道和文献回顾

Gary LU†, Inga GUREVICH, Binh T VO, Su S. CHEN
(Department of Hematopathology, University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA)

[关键词] 白血病，髓样；急性；基因重排；t(10;12) (q24;p13)

doi:10.3969/j.issn.1671-167X.2009.04.023

《国际公共卫生：疾病、计划、系统与政策》（第 2 版）中文版出版

由北京大学公共卫生学院郭新教授主编的《国际公共卫生：疾病、计划、系统与政策》（第 2 版）一书由化学工业出版社出版发行。该书是国际公共卫生领域的权威著作，发行以来受到世界各地读者的广泛欢迎，此次是该书的中文版首次在国内出版发行。

国际公共卫生是利用公共卫生的原理来解决各国、特别是发展中国家面临的健康问题和挑战，以及影响这些问题的一系列相关的全球性或地区性因素的科学。目前国际公共卫生的重点是在发展中国家，这些国家的人群死亡率和发病率高。卫生系统尚不完善，难以满足保护易感人人群健康的需要。改善人群健康状况需要全面了解其所处环境的社会、文化和经济特征，通过比较不同国家的公共卫生实践，可以获取宝贵的经验，不断提高各国的公共卫生管理水平。

该书全面系统地介绍了国际公共卫生的现状和背景。第 3~5 章介绍了联合国公共健康面临的三大传统挑战：传染性、入侵性疾病和营养。第 6~9 章介绍了公共卫生的重点领域：慢性病、伤害、精神疾病以及环境与健康。第 10 章介绍了全球性突发卫生事件对公共卫生带来的挑战。第 11 章主要从经济学的角度叙述了卫生系统的运行。第 12 章主要讨论了公共卫生管理与计划的有关问题。第 13 章讨论了影响发展中国家制定公共卫生政策的因素。第 14 章介绍了国际公共卫生全球合作的现状。最后一章系统地回顾了公共卫生如何影响国际公共卫生的发展。该书还附有许多实例，目其目的是针对相关章节出现的关键点和概念采用具体的事例进行说明。

本书不仅可供公共卫生及预防医学专业人员和学生参考，而且对于医疗卫生政策制订者也具有重要的参考价值。

（北京大学公共卫生学院）