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Case Report:

CD19-positive acute myeloblastic leukemia with trisomy 21 as a sole acquired karyotypic abnormality

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Abstract: We report that a 63-year-old Chinese female had acute myeloblastic leukemia (AML) in which trisomy 21 (+21) was found as the sole acquired karyotypic abnormality. The blasts were positive for myeloperoxidase, and the immunophenotype was positive for cluster of differentiation 19 (CD19), CD33, CD34, and human leukocyte antigens (HLA)-DR. The chromosomal analysis of bone marrow showed 47,XX,+21[2]/46,XX[18]. Fluorescent in situ hybridization (FISH) showed that three copies of AML1 were situated in separate chromosomes, and that t(8;21) was negative. The patient did not have any features of Down syndrome. A diagnosis of CD19-positive AML-M5 was established with trisomy 21 as a sole acquired karyotypic abnormality. The patient did not respond well to chemotherapy and died three months after the diagnosis. This is the first reported case of CD19-positive AML with trisomy 21 as the sole cytogenetic abnormality. The possible prognostic significance of the finding in AML with +21 as the sole acquired karyotypic abnormality was discussed.

Key words: Trisomy 21, Acute myeloid leukemia, Cluster of differentiation 19 (CD19)
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INTRODUCTION

Trisomy 21 (+21) is one of the five most frequent numerical abnormalities occurring in human neoplasms (Heim and Mitelman, 1986); however, in most cases, the extra chromosome 21 is present together with other numerical and/or structural changes. Acquired trisomy 21 is the single karyotypic abnormality in only 0.4% of human neoplasms, and the frequency was slightly lower in lymphoid than in myeloid malignancies (0.2% vs. 1.0%) (Mitelman *et al*., 1990). The incidence of trisomy 21 as a sole abnormality was between 0.3% and 0.6 % in all patients with acute myeloblastic leukemia (AML). Morphologically, AML with trisomy 21 as a sole abnormality preferentially shows M2 or M4 phenotype according to the French-American-British (FAB) classification (Gallego *et al*., 1997; Wan *et al*., 1999). Patients with this aberration appear to have a poor prognosis (Wan *et al*., 1999; Cortes *et al*., 1995; Wei *et al*., 1996), but the clinical and prognostic implications of this karyotypic abnormality in AML remain unclear. Recently, a small number of cases (Wei *et al*., 1996; Kondo *et al*., 2001; Yamamoto *et al*., 2002; Udayakumar *et al*., 2007) reported the relationship between AML with trisomy 21 as a sole acquired abnormality and expression of cluster of differentiation 7 (CD7), and reported that co-expression of CD7 is probably indicative of the very early stage at which the cell became malignant (Udayakumar *et al*., 2007). Here, we presented a case of CD19-positive AML with trisomy 21 as a sole acquired karyotypic abnormality and discussed its possible prognostic significance.

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CASE PRESENTATION

A 63-year-old Chinese woman was admitted to our hospital because of debilitation and fever for 3 months in July 2005. Physical examination showed no hepatosplenomegaly or lymphadenopathy. Peripheral blood counts showed hemoglobin 59 g/L, platelets 205×10^9 L^{-1}, and leukocytes 4.1×10^9 L^{-1} with 13% blasts, 7% neutrophils, 12% monocytes, and 68% lymphocytes. Bone marrow aspirate showed a hypercellular marrow with 68.5% monoblasts. The blasts were positive for myeloperoxidase, sudan black, and non-specific esterase, and were sensitive to fluoride inhibition. Therefore, the patient was diagnosed as AML FAB M5 (Fig.1). Immunophenotypic analysis by flow cytometry revealed that the blasts were positive for CD19 (38.56%), CD33 (20.05%), CD34 (75.29%), human leukocyte antigens (HLA)-DR (63.94%), but negative for other lymphoid markers including CD3, CD7, CD10, CD22, CD61, CD56, glycophorin A (GPA), CD79a, and cyCD3 (Fig.2, see Page 835).

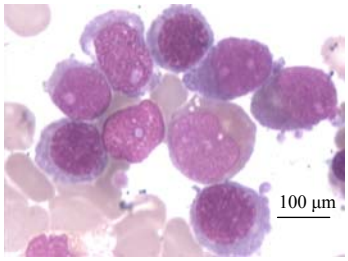

Fig.1 Bone marrow aspirate showed a hypercellular marrow with pleomorphic large monoblasts. The blasts were positive for myeloperoxidase, sudan black, and non-specific esterase, and were sensitive to fluoride inhibition

Conventional cytogenetics was performed at the time of diagnosis. Chromosomes were R-banded on unstimulated bone marrow cells after a 24-h culture. The cells were grown in RPMI 1640 (Sigma, Schnelldorf, Germany) supplemented with 20% (v/v) fetal bovine serum. At least 20 metaphases were analyzed. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature criteria (Shaffer and Tommerup, 2005). The remaining fixed cell pellets were stored at −20 °C for further fluorescent in situ hybridization (FISH) studies. Chromosome analysis of the bone marrow cells showed 47,XX,+21[2]/46,XX[18] (Fig.3).

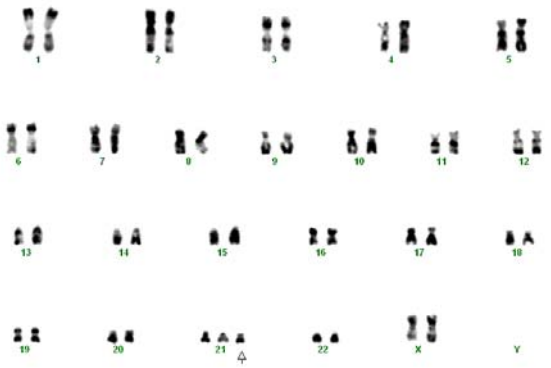

Fig.3 Karyotype of the patient showed 47,XX,+21 (arrow indicating the extra 21)

FISH analysis was performed on interphase/metaphase cells from the patient's bone marrow cells. Four hundred interphase/metaphase nuclei were counted for each hybridization assay. We used spectrum red- and spectrum green-conjugated dual-color break-apart probe specific for the AML1 gene (Cytocell, UK) to confirm the presence of extra chromosome 21, and found that three copies of AML1 were situated in separate chromosomes, which suggested chromosome 21 (Fig.4). In addition, Vysis dual-color, dual-fusion, locus-specific identifiers (LSI) AML1/ETO probes (Abbott Molecular/Vysis, Des Plaines, IL, USA) were used according to the manufacturer's recommendations. The LSI AML1 probe was labelled with spectrum green and the LSI ETO probe was labelled with spectrum orange. Three-green, two-orange signal pattern was detected, but AML1/ETO fusion signal was not detected.

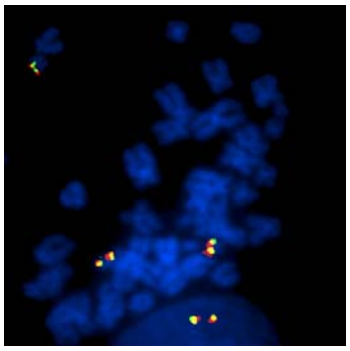

Fig.4 Spectrum red- and spectrum green-conjugated dual-color break-apart probe specific for the AML1 gene (Cytocell, UK) was used to confirm the presence of extra chromosome 21, and it was found that three copies of AML1 were situated in separate chromosomes, which suggested chromosome 21

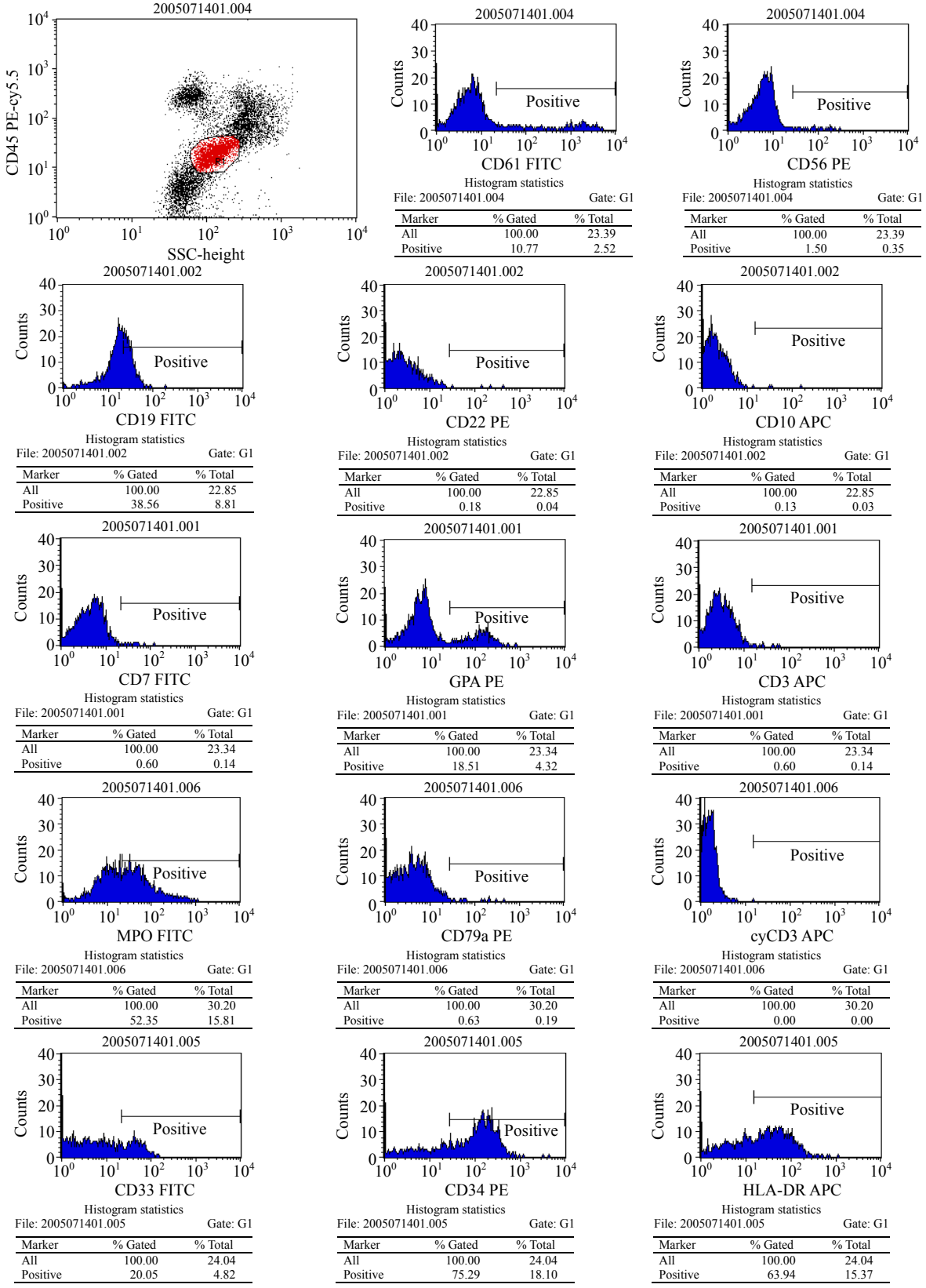

Fig.2 Flow cytometry showed that myeloblasts were positive (>20%) for CD19, CD33, CD34, HLA-DR, and MPO, but negative for other lymphoid markers including CD3, CD7, CD10, CD22, CD61, CD56, GPA, CD79a, and cyCD3. FITC: fluoresein-5-isothiocyanate; PE: phycoerythrin; APC: allophycocyanin; GPA: glycophorin A; MPO: myeloperoxidase; SSC: side scatter

We considered trisomy 21 to be acquired when the patient showed no phenotypic characteristics of Down syndrome, such as developmental retardation, brachycephaly, epicanthal folds, and speckled iris. The patient was diagnosed with AML-M5 with +21 as the sole acquired karyotypic abnormality, and treated with cytosine arabinoside (100 mg/m^2, continuous infusion, Days 1~7) and homoharringtonine (6 mg, Days 1~3). However, a repeated bone marrow showed a refractory state. The patient was then treated with cytosine arabinoside (100 mg/m^2, continuous infusion, Days 1~7) and etoposide (100 mg, Days 1~7). Unfortunately, she died of refractory leukemia after the second treatment, with survival duration of three months.

DISCUSSION

Trisomy 21 has been demonstrated to be a recurring cytogenetic abnormality in AML and myelodysplastic syndrome (MDS). Trisomy may contribute to leukemogenesis by a gene dosage effect; whereby the presence of an increased copy number of certain genes gives a cell survival advantage and hence neoplastic potential (Sacchi, 1992). Cells trisomic for chromosome 21 could be over-proliferating due to the enhanced expression of a tumourigenic gene (Shen *et al*., 1995). The incidence of trisomy 21 as a sole cytogenetic anomaly in the de novo AML varies from 0.3% to 0.6% and from 2% to 6.7%, when it is associated with other anomalies whose presence rather than trisomy 21 determines the clinical outcome (Mitelman, 1994; Mitelman *et al*., 1990; Cortes *et al*., 1995; Wei *et al*., 1996; Berger *et al*., 1987). Dewald *et al*.(1990) reported that trisomy 21 was observed as a sole acquired abnormality in 13 patients who had hematologic malignancies, of whom 12 had myeloid, including 5 myelodysplastic syndromes, 3 AML-M4, 3 AML-M2, and 1 AML-M7, and the remaining one had lymphoid leukemia. Many AML cases with trisomy 21 as a sole anomaly had been reported. Most of them were associated with AML-M2 or AML-M4, and the remaining cases included AML-M1, AML-M3, AML-M5, AML-M6, and AML-M7 (Table 1) (Dewald *et al*., 1990; Mitelman, 1994; Cortes *et al*., 1995; Gallego *et al*., 1997; Wei *et al*., 1996; Wan *et al*., 1999; Kondo *et al*., 2001; Yamamoto *et al*., 2002; Udayakumar *et al*., 2007). Trisomy 21 also had been documented in 16 patients with myelodysplastic syndromes, 6 chronic myelomonocytic leukemia, 2 acute lymphocytic leukemia (ALL), 2 bilineal leukemia, and 2 undifferentiated leukemia (Mitelman, 1994). By comparison, the most common hematological malignancies in patients with constitutional trisomy 21 (Down syndrome) are ALL and AML-M7 (Wan *et al*., 1999; Hasle *et al*., 2000). Here, we reported a new case of AML-M5 with trisomy 21 as the sole acquired karyotypic abnormality, which is in accordance with the observation reported in most cases that trisomy 21 was present along with normal clones (Gallego *et al*., 1997; Wan *et al*., 1999; Wei *et al*., 1996; Yamamoto *et al*., 2002; Dewald *et al*., 1990).

Table 1 Reports of trisomy 21 as a sole acquired abnormality in AML

References	Year	Country	Sex/age	FAB subtype	CD7	CD19	Outcome	Os (month)*
Wei *et al*.(1996)	1996	China	M/35	AML-M4	NA	NA	Died of refractory leukemia	9
		Taiwan, China	M/30	AML	NA	NA	Died of internal bleeding 3 d after chemotherapy	0.1
Gallego *et al*.(1997)	1997	Spain	M/86	AML-M5b	NA	NA	No treatment	NA
Wan *et al*.(1999)	1999	China	M/28	AML-M2	NA	NA	CR, received an ABMT	24
			F/78	AML-M4	NA	NA	No treatment	NA
Kondo *et al*.(2001)	2001	Japan	M/21	AML-M2	+	−	CR well at 4 months	NA
Yamamoto *et al*.(2002)	2002	Japan	M/49	AML-M2	+	−	CR, MDS 2 years later, leukemia 3 years later, died at 4 years	48
Udayakumar *et al*.(2007)	2007	Oman	M/24	AML-M2	+	−	CR, but relapsed soon	NA
Present case	2008	China	F/61	AML-M5b	−	+	Did not received CR	3

* When the patients were first diagnosed. M: male; F; female; NA: not available; +: positive; −: negative; CR: complete remission; FAB: French-American-British; Os: overall survival; ABMT: autologous bone marrow transplant; MDS: myelodysplastic syndrome

In children, trisomy 21 as the sole chromosomal abnormality in ALL represents a good prognosis both in patients with Down syndrome and non-Down syndrome (Watson *et al*., 1993; Raimondi *et al*., 1992). AML in Down syndrome also showed high responsiveness to chemotherapy with a good event-free survival (Ravindranath *et al*., 1992). In adults, the prognostic significance of trisomy 21 as the sole abnormality in AML remains unclear. A higher complete remission (CR) had previously been reported in AML with trisomy 21 (Arthur *et al*., 1989), but a more recent study (Cortes *et al*., 1995) showed that trisomy 21 in AML typically presented in conjunction with other cytogenetic changes, whose presence, rather than trisomy 21, determined the clinical outcome, and more studies (Wan *et al*., 1999; Cortes *et al*., 1995; Wei *et al*., 1996; Dewald *et al*., 1990) showed that AML patients with acquired trisomy 21 as sole abnormality had a poor prognosis. In our case, the patient also had poor prognosis, which is concordant with the poor prognosis reported in AML patients with trisomy 21 as the sole abnormality.

Expression of lymphoid antigen is common in AML. CD7 is the most commonly expressed lymphoid antigen in AML (11%~29%) (Drexler *et al*., 1993; Kita *et al*., 1993), and CD2 is expressed in 16%~21% and CD19 in 7%~14% (Bradstock *et al*., 1994; Ball *et al*., 1991). Recently, Kondo *et al*.(2001), Yamamoto *et al*.(2002), and Udayakumar *et al*.(2007) reported the relation between AML with trisomy 21 as a sole acquired abnormality and expression of CD7, suggesting that co-expression of CD7 was probably indicative of the very early stage, at which the cell became malignant (Udayakumar *et al*., 2007). The biological and clinical significance of CD7 expression in AML is not fully understood, although CD7-positive AML patients have a significantly lower response rate and poorer prognosis than CD7-negative AML patients (Drexler *et al*., 1993). It has been reported that co-expression of CD7 in cases of AML with acquired trisomy 21 is 12%~17% in the overall group of AML patients (Kita *et al*., 1993), and it is speculated that there are more cases of CD7-positive AML with acquired trisomy 21 (Kondo *et al*., 2001; Udayakumar *et al*., 2007). However, in our case, the patient did not express CD7, which was probably due to that most CD7-positive AML was associated with AML-M0, AML-M1, AML-M2, and AML-M4 (Saxena *et al*., 1998). CD19 is a B-lymphocyte marker, whose expression is associated with pediatric AML-M2 and the t(8;21) translocation (Schwarzinger *et al*., 1990). The biological and clinical significance of CD19 expression in AML is not clear, although it was reported that CD19-positive AML patients had a short survival (Solary *et al*., 1992). In our case, the CD19-positive AML patient, without t(8;21) translocation, had a poor prognosis, a 3-month survival, which is much shorter than the average survival duration of 12.5 months in our department for AML with trisomy 21 as a sole acquired abnormality, which did not express lymphocytic marker. We, therefore, speculate that the AML patients with trisomy 21 as a sole acquired abnormality who expressed lymphocytic markers probably have a poor prognosis. However, the role of lymphoid antigen expression in response to treatment in AML patients with trisomy 21 needs to be further studied.

CONCLUSION

In this case, AML was found to have trisomy 21 as the sole acquired karyotypic abnormality. The patient did not respond well to chemotherapy and died three months after the diagnosis. More cases of AML with sole acquired trisomy 21 need to be immunophenotypically analyzed to assess the prognostic significance of lymphoid antigen expression.

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