Acute lymphoblastic leukemia in a patient with congenital neutropenia without G-CSF-R and ELA2 mutations

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TO THE EDITOR

Severe congenital neutropenia (SCN) is a hematological disorder characterized by profound neutropenia (<200/μl) and a maturation arrest of myelopoiesis in the bone marrow. The disease presents with a variable inheritance. In a subset of cases with SCN, mutations are found in granulocyte colony-stimulating factor receptor (G-CSF-R) gene resulting in truncation of distal region of the receptor protein involved in maturation signaling.[1] Novel mutations in the extracellular domain of the G-CSF-R have been reported in patients with SCN hypo/nonresponsive to G-CSF treatment.[2] Mutations of G-CSF-R gene strongly correlate with predisposition to acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) in SCN (Germeshausen *et al*. *Blood* 1999; **94**: 45 (abstract)).[3] Recently, a variety of mutations in the gene encoding the serine protease neutrophil elastase (ELA2) have been demonstrated in majority of patients with SCN (Aprikyan *et al*. *Blood* 2000; **96**: 445 (abstract)).[4] G-CSF-R mutation has been associated in most patients with ELA2 mutations that show evolution into leukemia.[5] Development of acute lymphoblastic leukemia (ALL) in patients with SCN has been extremely rare, and to our knowledge, only two cases who carried G-CSF-R gene mutations have been described.[5,6] Here we report a 7-month-old girl with SCN who transformed into CALLA+ B-cell ALL.

Case report

The patient was diagnosed as SCN at the age of 5 months. She was the first born child of parents who were second-degree relatives. There was no family history for an increased susceptibility to infections. The patient had a history of early-onset frequent episodes of potentially life-threatening bacterial infections. Her hemoglobin (Hb) was 102×10^9/l, white blood cell (WBC) count was 6.3×10^9/l and differential count was 1% neutrophils (absolute neutrophil count: 0.063×10^9/l), 30% monocytes, 63% lymphocytes and 6% eosinophils. Her bone marrow aspiration smears revealed apparent arrest at promyelocyte serie, despite presence of very few myelocytes. The immunological tests were within normal limits except elevation in IgG, which was attributed to recurrent bacterial infections. At 7 months of age, before the initiation of G-CSF treatment her Hb was 92×10^9/l, WBC 7.2×10^9/l and her peripheral blood smear revealed 20% lymphoblasts. Viral markers like parvo virus, Epstein–Barr virus, cytomegalovirus, hepatitis A, hepatitis B and hepatitis C were negative. Bone marrow and flow cytometric examination revealed 40–50% lymphoblasts consistent with CALLA+ B-cell ALL (pre-B): CD19 (40%), CD20 (29%), CD22 (40%), CD10 (38%), HLA-DR (39%). The patient attained remission following induction therapy of St Jude Total XI Study ALL chemotherapy protocol. After remission induction therapy, her neutrophil percentage in the peripheral blood kept between 0 and 2%. She responded to G-CSF (10 μg/kg) administration with an increase in absolute neutrophil count up to 4900/μl, but after cessation of G-CSF, neutrophil percentage tended to fall to 1% in a short time. Unfortunately, the patient died because of septicemia during maintenance therapy.

Correspondence: Dr S Yetgin, Department of Pediatric Hematology, Hacettepe University, İhsan Doğramacı Children's Hospital, 06100 Ankara, Turkey; Fax: +90 312 324 1681;
E-mail: yetgins@hacettepe.edu.tr or yetgins@superonline.com
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Genetic analysis

ELA2 mutation analysis

Genomic DNA was extracted from the peripheral blood, as described previously.[7] Five fragments of DNA covering all five exons and the promoter region of ELA2 and at least 15 bases of the flanking regions were amplified using PCR and sequenced directly. The sequence analysis of ELA2 gene did not reveal any mutation. The only nucleotide change observed was N101N polymorphic change, which did not cause any change in the amino-acid code.

G-CSF-R analysis

A fragment encompassing the previously reported nonsense mutations in patients with congenital neutropenia (nucleotides 2384–2429, accession no. M59818) was amplified by PCR. The PCR products were sequenced after subcloning. A G-CSF-R mutation in the region known to be susceptible for the acquisition of somatic point mutations in SCN patients was not detected.

Discussion

While patients with SCN have a predisposition for AML and MDS, only two patients with SCN who developed secondary ALL were reported.[5,6] The mechanism of leukomogenesis in SCN is unclear. The presence of point mutations in G-CSF-R in SCN/ALL patients comparable to those detected in SCN patients who developed secondary AML suggests that G-CSF-R mutation is involved in leukomogenesis. Tyrosine residues are crucial in G-CSF signaling. On the other hand, although most of the SCN patients with G-CSF-R mutation developed leukemia, there are also SCN patients with G-CSF-R mutations who have not yet progressed to leukemia and furthermore, there are SCN/AML patients who do not harbor such a mutation (Germeshausen *et al*. *Blood* 1999; **94**: 45 (abstract)).[5] In one of the two patients who progressed to ALL,[5] the mutational deletion included all of the tyrosine residues. It was speculated that G-CSF-R mutations presented in SCN are heterogenous and complex at the side of stem cell, intracellular signaling and leukomogenesis.

Our patient is the third case with ALL secondary to SCN but it is the first SCN/ALL patient who lacks a G-CSF-R mutation.

Recently, ELA2 gene mutations in SCN have been studied extensively and were reported to be responsible for neutropenia in some of these patients.[8] Patients with SCN display a great

heterogeneity in occurrence and type of ELA2 gene mutation, and different molecular alterations seem to yield a different clinical phenotype. Neither the variation in the expression of neutropenia nor the transformation to leukemia in SCN has been clearly explained by ELA2 mutation so far.[8] Despite the high incidence of ELA2 mutation in patients with cyclic neutropenia, up to now none of the cyclic neutropenia patients have shown malignant transformation.[4]

All these contradictory points mentioned above suggest that neither SCN profile nor transformation to leukemia can be explained by the occurrence of G-CSF-R or ELA2 mutations alone. Additional genetic factors, including the genes that are directly related to the hematopoietic hierarchy, or modifier genes that affect disease expression seem to be involved in leukemic progression in SCN.

It could be possible that presence of an unknown mutation in our patient or transformation of the disease to ALL unexpectedly may be coincidental. We think that an unknown genetic alteration may have led to both SCN and then transformation to ALL within a period as short as 7 months.

S Yetgin[1]
M Germeshausen[2]
I Touw[3]
A Koç[1]
L Olcay[1]

[1]*Department of Pediatric Hematology, Hacettepe University, İhsan Doğramacı Children's Hospital, Ankara, Turkey;*
[2]*Department of Pediatric Hematology and Oncology, Medical School Hannover, Germany; and*
[3]*Institute of Hematology, Erasmus University, Rotterdam, Netherlands*

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