Case Report

Acta Haematol 2012;128:73–76
DOI: 10.1159/000338220

Received: January 9, 2012
Accepted after revision: March 8, 2012
Published online: June 6, 2012

Higher Expression of the Novel Gene Upregulated Gene 4 in Two Acute Lymphoblastic Leukemia Patients with Poor Prednisolone Response

Yesim Oymak[a] Yavuz Dodurga[b] Aysen Turedi[c] Yontem Yaman[d]
Gulcihan Ozek[d] Ozgur Carti[d] Burcak Tatlı Gunes[d] Esin Erbudak[d]
Ergul Berber[f] Cıgır Biray Avci[e] Canan Vergin[d]

[a]Department of Pediatric Hematology, Harran University, Sanliurfa, [b]Department of Medical Biology and Genetics, Medical Faculty, Pamukkale University, Denizli, [c]Diyarbakir Children's Hospital, Diyarbakir, [d]Department of Pediatric Hematology, Behcet Uz Children's Government Hospital, and [e]Department of Medical Biology and Genetics, Medical Faculty, Ege University, Izmir, and [f]Department of Molecular Biology and Genetics, Arel University, Istanbul, Turkey

Key Words
Acute lymphoblastic leukemia · Leukemogenesis · Poor prednisolone response · *URG4*

Abstract
Elucidation of the molecular mechanisms of leukemogenesis is important for a better understanding of the prognosis of acute lymphoblastic leukemia (ALL). Studies have shown that the expression of upregulated gene 4 *(URG4),* which promotes cell growth and survival, is increased in different types of carcinomas including hepatocellular carcinoma, gastric cancer and osteosarcoma. Similarly, higher expression of *URG4* and cyclin D1 gene might promote proliferation of the blast cells by causing escape from the G1 checkpoint and entry into the S phase. This study reports the high expression level of *URG4* in 2 high-risk ALL patients for the first time in the literature. In conclusion, the higher expression of *URG4* in our 2 patients suggests that *URG4* might be involved in leukemogenesis. Future studies with a large number of high-risk ALL patients and cell culture studies are needed to demonstrate the exact role of *URG4* in leukemogenesis.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignant disease. Variations in the expression level of the cell cycle genes including cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors have been associated with leukemia [1–3].

Recently, upregulated gene 4 *(URG4)* located on chromosome 7p13 has been implicated in tumorigenesis. For example, it was demonstrated that HBxAg-positive hepatocellular carcinoma cells have a strong expression of *URG4* [4]. Moreover, the high level of *URG4* expression in osteosarcoma tissues was correlated with proliferative activity of osteosarcoma cells [5]. It was suggested that *URG4* may play a role in the development of carcinoma by regulating the expression of the cyclin D1 gene *(CCND1)* [5]. *URG4* has also been proposed as a therapeutic target in the treatment of carcinomas such as gastric cancer due to its implication in carcinogenesis [6].

Although research in the understanding of the molecular basis of ALL has been going on, the molecular pathogenesis of leukemia has not yet been elucidated. However, more precise risk factors have been identified

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0001–5792/12/1282–0073$38.00/0

Accessible online at:
www.karger.com/aha

Dr. Yavuz Dodurga
Pamukkale University Department of Medical Biology and Genetics
Kinikli Kampusu Morfoloji Binasi Kat: 3
TR–20070 Denizli (Turkey)
Tel. +90 258 296 2534, E-Mail yavuzdodurga@gmail.com

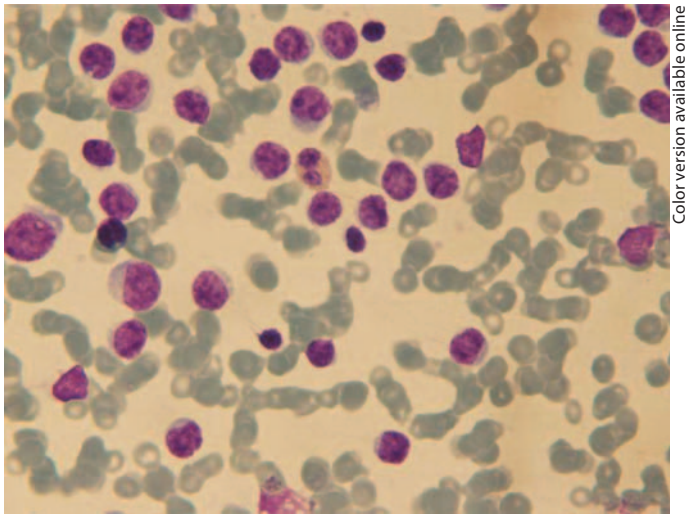

Fig. 1. Bone marrow aspirate smear of case 1. Bone marrow aspiration shows diffuse L1 type blastic infiltration. ×100.

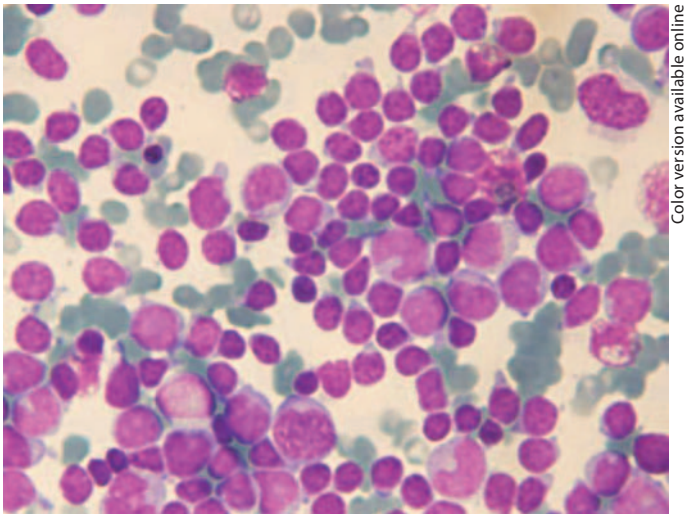

Fig. 2. Bone marrow aspirate smear of case 2. The bone marrow shows blast cells that are characteristic of ALL. ×100.

in childhood leukemia. Prednisolone response on the 8th day of induction therapy is the earliest one for the Berlin-Frankfurt-Munich (BFM) group. Patients with a poor prednisolone response on the 8th day of treatment are classified as high-risk patients in the BMF group.

In the present study, we report 2 cases of high-risk ALL patients according to the BFM criteria with a higher expression of *CCND1* and *URG4*. This is the first report showing *URG4* upregulation in 2 ALL patients with poor prednisolone response.

Case Reports

Case 1

A 12-year-old girl presented to the Hematology Clinic of Dr. Behcet Uz Children's Hospital because of high fever and weight loss. She had splenomegaly and her peripheral blood sample profile showed 12.7 g/dl hemoglobin, 146.0 × 10^9/l platelets and 6.4 × 10^9/l leukocytes with 35% of blast cells. Moreover, L1 type blastic infiltration was observed in the bone marrow aspiration smear (fig. 1). The patient was diagnosed as a pre-B-cell ALL patient according to flow cytometry analysis. Her central nervous system was affected by dural enhancement in the supratentorial compartments, which was isointense on T_1-weighted and hyperintense on T_2-weighted MR images. There were four lymphocytes in the cerebrospinal fluid count (by the Nageotte chamber method). Cytogenetic analysis, fluorescence in situ hybridization and RT-PCR analysis of bone marrow did not show any abnormality.

Initially, chemotherapy of the BFM-2000 protocol with intermediate-risk group was applied. However, she had a poor prednisolone response with 1.178 × 10^9/l absolute blast count on the 8th day of treatment. Therefore, she was reclassified as a high-risk group patient. Although 98% of the blast cells were observed on the 15th day of bone marrow treatment, she was in remission at the end of the remission induction therapy. She underwent hematopoietic stem cell transplantation from a human leukocyte antigen-identical older sister after the first three high-risk blocks and she has been leukemia free for 2 years.

Case 2

Case 2 was an 8-year-old boy with a 2-week history of increasing fatigue, weakness and pallor. Physical examination revealed hepatosplenomegaly. His blood counts indicated leukocytosis (172.0 × 10^9/l), anemia (hemoglobin 3 g/dl) and thrombocytopenia (22.0 × 10^9/l). The peripheral blood analysis and bone marrow analysis showed L1 type blasts (55 and 65% blast cells, respectively) which is the characteristic feature of ALL patients (fig. 2). The blast cells coexpressed T-cell markers and CD13. Although hypodiploidy was observed during cytogenetic analysis, fluorescence in situ hybridization and RT-PCR analysis did not show any abnormality. The patient was classified as an intermediate-risk patient due to his age, his high leukocyte count and his T-cell immune phenotype, and thus, the BFM-2000 protocol was applied. The absolute blast count of the patient on day 8 was 70.40 × 10^9/l and bone marrow on day 15 showed 3% blast cells. At bone marrow evaluation on day 33, the patient was in remission. His risk group changed to high risk due to the poor prednisolone response on the 8th day. The patient is still alive and receiving chemotherapy due to the absence of a matching donor.

Method

Quantitative RT-PCR

In order to evaluate the molecular pathogenesis of leukemia in our 2 patients, the expression level of *URG4* was analyzed. Bone marrow samples from the 2 patients and 6 controls without ma-

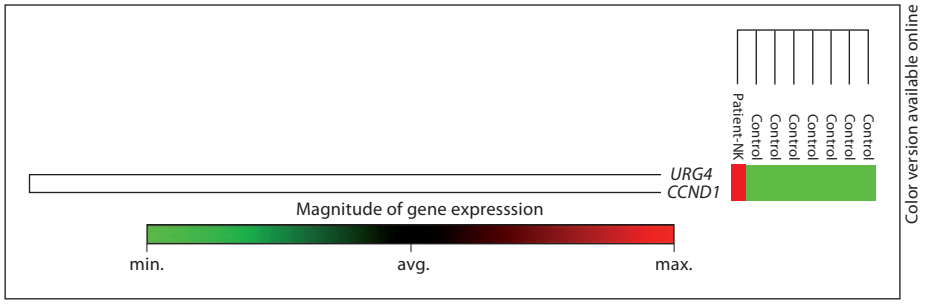

Fig. 3. Expression levels of *URG4* and *CCND1* for case 1. Min. = Minimum; avg. = average; max. = maximum; NK = patient name ID.

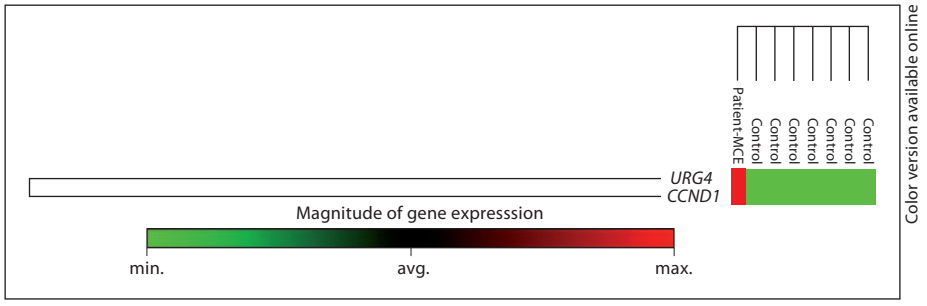

Fig. 4. Expression levels of *URG4* and *CCND1* for case 2. Min. = Minimum; avg. = average; max. = maximum; MCE = patient name ID.

lignancy were obtained for molecular analysis. Control bone marrow samples were taken during routine screening for diagnostic purposes, and the informed consent of the patients was obtained. The expression levels of *URG4* and *CCND1* were analyzed by quantitative RT-PCR method with the Light Cycler 480 Real Time PCR system. Fifty microliters (50–200 ng) of total RNA was isolated from the bone marrow samples by using the RT^2 First Strand Kit (Roche Diagnostics, Germany) according to the manufacturer's manual. Glyceraldehyde-3-phosphate dehydrogenase ('housekeeping' gene) was used as an internal control in the RT-PCR. The primer sequences are given in table 1. The results were analyzed by using the ΔΔCT method and quantitated with Light Cycler® 480 Quantification Software.

Table 1. Primers used for RT-PCR

Primer name	Sequence
URG4	F: 5′-CGGGAGATGGGACAGTTTTA-3′
URG4	R: 5′-CATGGTGTTGAGGAGTGTGG-3′
CCND1	F: 5′-AGCTCCTGTGCTGCGAAGTGGAAAC-3′
CCND1	R: 5′-AGTGTTCAATGAAATCGTGCGGGGT-3′
GAPDH	F: 5′-CCCCACACACATGCACTTACC-3′
GAPDH	R: 5′-CCTAGTCCCAGGGCTTTGATT-3′

F = Forward; R = reverse; GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

Results

Previous studies have implicated a functional role for *URG4* in cell cycle control and tumor development. In the present study, increased expression of *URG4* was demonstrated in 2 patients diagnosed with childhood ALL. The expression level of *URG4* and *CCND1* in the patients was analyzed by quantitative RT-PCR method and compared with the expression level in the control patients without malignancy. The expression of *URG4* (fig. 3) and *CCND1* (fig. 4) was increased in both patients. Case 1 had a 6,623.3- and a 14,050.5-fold increase in *URG4* and *CCND1* expression, respectively. Similarly, case 2 showed a 544.3- and a 3,133.0-fold increase in *URG4* and *CCND1* expression, respectively (table 2).

Table 2. Change in *URG4* and *CCND1* expression in 2 patients (according to the comparison of cases and control patients)

	Case 1		Case 2	
Gene symbol	*URG4*	*CCND1*	*URG4*	*CCND1*
Fold change	6,623.3	14,050.5	544.3	3,133.0

Discussion

Genome-wide approaches have contributed to a better understanding of tumorigenesis by identifying the oncogenes and have also delineated novel genetic alterations

that are associated with prognosis. Recent studies have implicated that the novel oncogene *URG4* has a role in tumorigenesis.

We used the BFM study group protocol to classify the patients. According to the BFM study group protocol, poor prednisolone response on the 8th day of therapy characterizes patients as high-risk patients. Accordingly, case 1 and case 2 are classified as high-risk patients with 1.178×10^9/l and 70.400×10^9/l peripheral blast on the 8th day of therapy, respectively.

In the present study, higher expression of *URG4* was demonstrated in 2 high-risk ALL patients for the first time. A 6,623.3- and a 544.3-fold increase in *URG4* expression was observed together with an increased expression of *CCDN1*.

CDKs and cyclins are important components of the eukaryotic cell division cycle. D-type cyclins are regulatory partners of G1-CDKs which are required for entry into the S phase of the cell cycle. The cyclin protein level is important for cell proliferation since the activity of CDKs depends on the availability of cyclins [7]. Studies have shown that an abnormal increase in the cellular *CCND1* level can cause malignant cell transformation and resistance to apoptosis, thus contributing to the resistance of several tumor cells to chemotherapeutic agents [8–11]. In addition, studies have indicated that expression of *URG4* promotes growth factor-independent survival [12] and *URG4* has a role in tumorigenesis by affecting the *CCND1* level through bypassing the checkpoint during the G1 to S phase transition [5, 12]. Therefore, we suggest that *URG4* might act via *CCND1* and contribute to uncontrolled proliferation of blasts in the 2 leukemia cases presented here, since overexpression of both *CCND1* and *URG4* were observed.

Another distinguishing feature of our 2 patients was poor prednisolone response which is a well-known prognostic feature for childhood ALL. The high level of *URG4* and *CCND1* in addition to the high blast count on the 8th day might implicate the presence of an ALL subtype.

In conclusion, this study provides the first clue that *URG4* could be implicated in the molecular pathogenesis of leukemia. Future studies with a large number of patients are needed to reveal the exact role of *URG4* in the molecular pathogenesis of leukemia.

References

1 Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, Visvader J, Weissman IL, Wahl GM: Cancer stem cells – perspectives on current status and future directions: AACR workshop on cancer stem cells. Cancer Res 2006;66:9339–9344.

2 Benekli M, Baer MR, Baumann H, Wetzler M: Signal transducer and activator of transcription proteins in leukemias. Blood 2003; 101:2940–2954.

3 Gesbert F, Griffin JD: Bcr/Abl activates transcription of the Bcl-X gene through STAT5. Blood 2000;96:2269–2276.

4 Satiroglu-Tufan NL, Dodurga Y, Gok D, Cetinkaya A, Feitelson MA: RNA interference mediated *URG4* gene silencing diminishes cyclin D1 mRNA expression in HepG2 cells. Genet Mol Res 2010;9:1557–1567.

5 Huang J, Zhu B, Lu L, Lian Z, Wang Y, Yang X, Satiroglu-Tufan NL, Liu J, Luo Z: The expression of novel gene *URG4* in osteosarcoma: correlation with patients' prognosis. Pathology 2009;41:149–154.

6 Song J, Xie H, Lian Z, Yang G, Du R, Du Y, Zou X, Jin H, Gao J, Liu J, Fan D: Enhanced cell survival of gastric cancer cells by a novel gene URG4. Neoplasia 2006;8:995–1002.

7 Pardee AB: A restriction point for control of normal animal cell proliferation. Proc Natl Acad Sci USA 1974;71:1286–1290.

8 Shintani M, Okazaki A, Masuda T, Kawada M, Ishizuka M, Doki Y, Weinstein IB, Imoto M: Overexpression of cyclin DI contributes to malignant properties of esophageal tumor cells by increasing VEGF production and decreasing Fas expression. Anticancer Res 2002;22:639–647.

9 Harada H, Nakagawa K, Iwata S, Saito M, Kumon Y, Sakaki S, Sato K, Hamada K: Restoration of wild-type p16 down-regulates vascular endothelial growth factor expression and inhibits angiogenesis in human gliomas. Cancer Res 1999;59:3783–3789.

10 Kornmann M, Arber N, Korc M: Inhibition of basal and mitogen-stimulated pancreatic cancer cell growth by CCND1 antisense is associated with loss of tumorigenicity and potentiation of cytotoxicity to cisplatinum. J Clin Invest 1998;101:344–352.

11 Warenius HM, Seabra LA, Maw P: Sensitivity to cisdiamminedichloroplatinum in human cancer cells is related to expression of CCND1 but not c-raf-1 protein. Int J Cancer 1996;67:224–231.

12 Tufan NL, Lian Z, Liu J, Pan J, Arbuthnot P, Kew M, Clayton MM, Zhu M, Feitelson MA: Hepatitis Bx antigen stimulates expression of a novel cellular gene, *URG4,* that promotes hepatocellular growth and survival. Neoplasia 2002;4:355–368.